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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

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VOLUME XXXVII — *MARCH, 1948* — NUMBER 3 — CONSECUTIVE NO. 6

An Investigation of *Piscidia Erythrina* (Jamaica Dogwood)*

By CHRISTOPHER H. COSTELLO and CALVIN L. BUTLER†

The chemical, pharmacological, and clinical history of Jamaica dogwood is reviewed. Preparations of all parts of Piscidia were found to exert appreciable uterine depressant action both *in vitro* and *in vivo* on laboratory animals. The root bark extracts were the most potent, and petroleum ether was found to be the most effective solvent for the depressant principle. The drug is apparently nontoxic to rats fed massive oral doses.

WHILE investigating the actions of various drugs employed in uterine sedative formulas (1, 2), it was found that *Piscidia erythrina* Linn., commonly known as Jamaica Dogwood, was a potent depressant in our hands on the uteri *in vitro* and *in vivo* of various laboratory animals and was worthy of further investigation. The present report concerns the study of the uterine activity of this plant, and the chemical work done for the purpose of tracing the potent substances, with a view to isolation. •The latter was not intended to be complete.

HISTORY

Jamaica Dogwood is a leguminous tree, native to the West Indies and the adjacent mainland, and also found in Florida, Texas, Mexico, and the northern portion of South America (3). The plant has a long history of use by the natives of Jamaica as a hypnotic and fish poison. The taste or edibility of the fish are in no way affected by this process (3, 4). It is also an ingredient of the Mexican native sedative tea called "Sinicuichi" together with two other plants (5).

Although the drug has never been official in any American compendium, it has appeared in several European pharmacopœias. Piscidia bark and its fluidextract were admitted in 1930 to the Supplement of the German Drug Book, Vol. 5, 1930 (4).

The chemical investigation originates with Hart (6) who, in 1883, obtained a neutral crystalline substance on treating the fluidextract with slaked lime. He believed this to be the active principle and named it piscidin. This was credited by Berberich (7) as having the formula $C_{29}H_{34}O_8$, melting at 192°.

Freer and Clover (8) have shown that the substance known as piscidin is a mixture of two glycosides. The first of these had a composition of $C_{23}H_{20}O_7$, forming colorless, highly refracting rectangular crystals, melting at 201°. The second had a composition of $C_{22}H_{18}O_6$, forming fine yellow

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† We wish to acknowledge the technical assistance of Lucille E. Mullen and Ursula E. Gassiraro.

needles, melting at 216°. Piscidic acid, having a composition of $H_2 \cdot C_{11}H_{10}O_7$ was reported in the aqueous extract, forming acicular crystals melting at 182–185°.

An exhaustive chemical investigation of the bark was conducted by Danckwortt and Schütte (9) in 1934. They obtained from the petroleum ether extract a crystalline alcohol of the paraffin group ($C_{17}H_{36}O$, m. p. 79°) and a crystalline monohydric alcohol belonging to the sterols ($C_{27}H_{48}O_2$, m. p. 145°). From the ether extract was obtained two crystalline substances, $C_{15}H_{14}O(OH)_2(OCH)_3CO_2H$, m. p. 158°, and an amorphous substance of undetermined composition, m. p. 70°. A characteristic constituent was found to be a water-soluble glycoside of saponin character, yielding glucose and galactose. Both acidified alcohol, and trichloroethylene extracted an alkaloidal substance, m. p. 86–87°, as a yellowish white amorphous powder. The latter employed the stem bark, whereas the previous authors used the root bark.

The earliest work on the pharmacodynamics of Jamaica Dogwood appears to be that of Ott (10, 11) and Nagle (12) who both found that it had narcotic properties, caused dilation of the pupils, increased secretion of sweat, and caused rise of blood pressure in lower animals.

Pharmacological research by Pellocani (13) and Cushny (14) showed that the mixture designated as piscidin could not be the active principle.

Pilcher and his associates (15), in 1916, found that Piscidia diminished the contractions of the excised intestinal strip of the rabbit and the excised uterus of the guinea pig but did not affect arterial smooth muscle. The action of the drug on lessening the amplitude and rate of contractions of the isolated guinea pig uterus was confirmed by Macht (16).

Pittenger and E'We (17) reported that the fluidextract administered orally to dogs had an action similar to cannabis but required approximately seventeen times as much to produce the same degree of incoordination. Piscidin obtained from the fluidextract represented 55% to 62.5% of its activity. This is in contradiction to the earlier reports of Pellocani and Cushny.

Pharmacological investigation of the saponin isolated by Schütte from the tree bark was conducted by Kofler (18). The lethal dose by intravenous injection was 0.075 mg. per Gm. of mouse. Oral doses up to 1.5 mg. per Gm. of mouse, however, had no effect.

An alcohol-soluble principle isolated from the petroleum ether extract was found by Hauschild (4) to be toxic to fish in dilutions of 1:80,000,000. The substance was strongly poisonous to rats and rabbits when administered parenterally, causing death by respiratory paralysis, but was nontoxic when administered orally in doses up to 5 mg. per Kg.

The effect of this drug on man as a remedy has not as yet been fully determined. Various investigators have reported good results from its use as an anodyne in neuralgia, nervous insomnia, cough, and dysmenorrhea. Ott (10, 11) first recommended Jamaica Dogwood in the treatment of chronic cough. Firth (19), in 1883, reported excellent results with the drug in delirium tremens. Payne (20) made a similar report. In the same year, Seifert (21) tried it clinically at the Julius Hospital in Vienna, and found it to be valuable in phthisis, causing a diminution of night sweating and also of cough, without any side effects. Later, Hamilton (22) called the attention of the profession to Jamaica Dogwood as a powerful sedative and analgesic, without the side effects that follow the use of opium and its derivatives. Peyer (23) used the bark as a sedative in the form of a tea. The most recent clinical contribution is that of LeClerc (24) who reported its value in the relief of neuralgia and whooping cough.

Earlier investigators report the drug as consisting of the root bark. Upon examination of the commercial product and correspondence with well-informed sources in Jamaica, it proved to be the stem bark, a by-product of lumbering operations. The commercial product used in this investigation was supplied by S. B. Penick and Company in 1947. Material collected locally from all parts of the plant was supplied by Mr. Arthur R. Marshall of Miami, Florida, and Mr. Cecil B. Facey, Kingston, Jamaica. All material used was authenticated pharmacognostically by Dr. Heber W. Youngken, Boston.

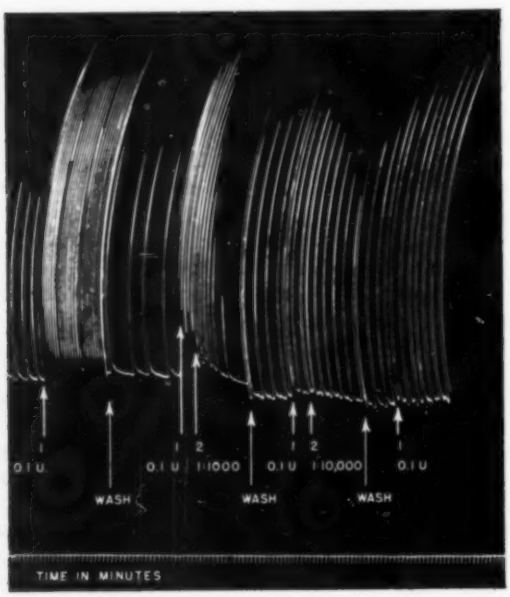
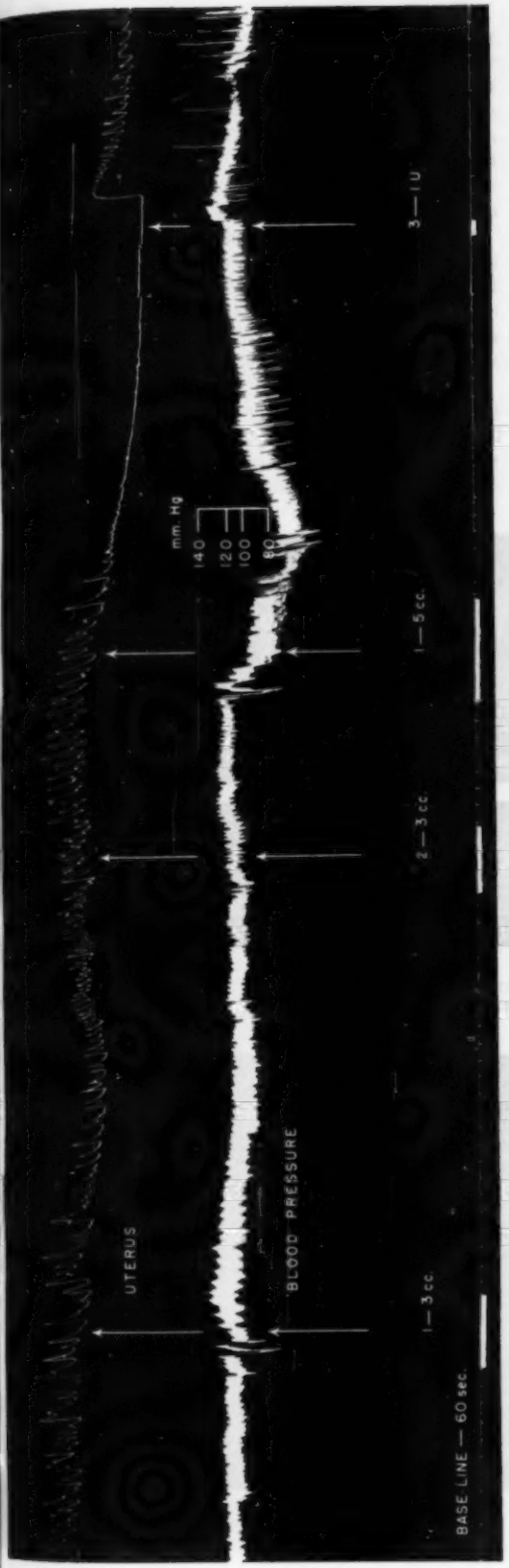


Fig. 1.—Effect on rat (anestrus), isolated uterus, left horn, *in vitro*. Drugs: 1, Pitocin; 2, Piscidia fluidextract, dealcoholized, buffered, not filtered.

EXPERIMENTAL

Pharmacological Methods.—Experiments upon isolated tissues were carried on according to the Magnus method as described by Burn (25). The isolated uterus of the rat was used by preference



since results were more easily reproducible and the various stages of the estrus cycle could be easily determined. The majority of the *in vivo* studies were based upon modification of the Barbour method. Tracings were made simultaneously of uterine contractions, blood pressure, and respiration. Drugs were given intravenously. A number of experiments employing the intrauterine balloon method were also made upon the *Rhesus macacus* monkey. This method is a modification of those employed by Bickers (26) and Woodbury, *et al.* (27).

Solutions used *in vitro* and *in vivo* were usually dealcoholized, buffered, and filtered. However, in certain cases *in vitro*, this procedure was omitted, since we found by experiment that the very small volumes of the various solvents for the drug showed no effect upon the tissue. Detailed procedures are described in previous papers (2, 28).

Fluidextract.—The fluidextract was prepared from commercial bark by the U. S. P. method type process A using 38.5% alcohol as a menstruum, and was first dealcoholized and buffered to *p*H 7.5 before use in any experiment. In a series of twenty *in vitro* experiments with the fluidextract of Piscidia, effective sedation was shown using dilutions ranging from 1:17,500 to 1:20,000. This was likewise found effective in reducing the spasmolytic effect of Pitocin, dilutions of from 1:1000 to 1:10,000 usually materially decreasing the stimulant effect of 0.1 unit (Fig. 1).

In the *in vivo* tests, uterine contractions of the cat were usually reduced using 3 to 5 cc. of the fluidextract. Blood pressure showed irregular drops and rises, especially during injection, while respiration was variable (Fig. 2).

Using the intrauterine balloon method on the monkey, 3 cc. of the fluidextract was found to be inhibiting to uterine motility (Fig. 3).

Alcoholic Extract.—For the purpose of a more complete examination, 10 Kg. of ground commercial bark was exhausted with alcohol in a Soxhlet apparatus. The resulting liquid was freed from alcohol by vacuum distillation to give 3.17% of a dark, granular material. In terms of the powdered drug extracted, the alcoholic extract was found to be effective *in vitro* in a concentration of 1:50,000. Computed in terms of the yield of extract, the concentration is 1:1,600,000.

The dried alcoholic extract was distilled with steam for over six hours, cooled, and filtered.

Volatile Oil.—The distillate yielded a volatile oil collected by means of ether, which amounted to 0.011% of the dried bark. The oil was amber in color with a pungent taste and an acrid odor. Insufficient material was available for further chemical study. Although weakly active, this fraction does

Fig. 2.—Effect on cat, 2.04 Kg. (in estrus), *in vivo*. Drugs (intravenous): 1, fluidextract, buffered, filtered; 2, Alcohol 38.5%; 3, Pitocin.

not constitute the active principle of the bark. In a series of three *in vitro* experiments, the oil showed sedation at a concentration of 1:500 of the drug or 1:100,000 of the oil itself.

Resin.—The residue from distillation of the alcoholic extract contained a physiologically active brown resin amounting to 1.96% of the bark. The resin obtained from commercial bark showed inhibition of uterine activity *in vitro* in a concentration of 1:10,000 in terms of the drug. The resin from the root bark was considerably more potent, producing sedation at a concentration of 1:75,000.

Aqueous Solution.—The filtrate from the resin was completely precipitated with lead acetate solution. The precipitate was suspended in water and decomposed with disodium phosphate; the mixture was filtered and evaporated to dryness. The brown semisolid amounted to 0.073% of the bark and reduced Fehling's solution only after hydrolysis, but was found to be pharmacologically inert.

The filtrate from the lead acetate precipitation yielded an inactive residue amounting to 1.91% of the bark. This was redissolved, and the solution precipitated completely with lead subacetate. This precipitate was also suspended in water and decomposed with disodium phosphate; the mixture was filtered and evaporated to dryness. The brown semisolid obtained amounted to 0.12% of the bark and reduced Fehling's solution only after hydrolysis.

Since only the resin had significant activity on the uterus, and this activity was approximately one-fifth that of the alcoholic extract from which it was derived, one is justified in concluding that the greater portion of the active principle was lost during the process of separation employed.

Successive Extraction.—In order to more closely trace the uterine acting principles with a minimum of loss by chemical methods, 1000 Gm. of ground commercial bark was exhausted in a Soxhlet apparatus employing a series of different solvents in succession.

Fig. 3.—Effect on monkey, 4.88 Kg. (in estrus), intrauterine balloon method. Drug (intravenous): Piscidia fluidextract, dealcoholized, buffered, filtered.

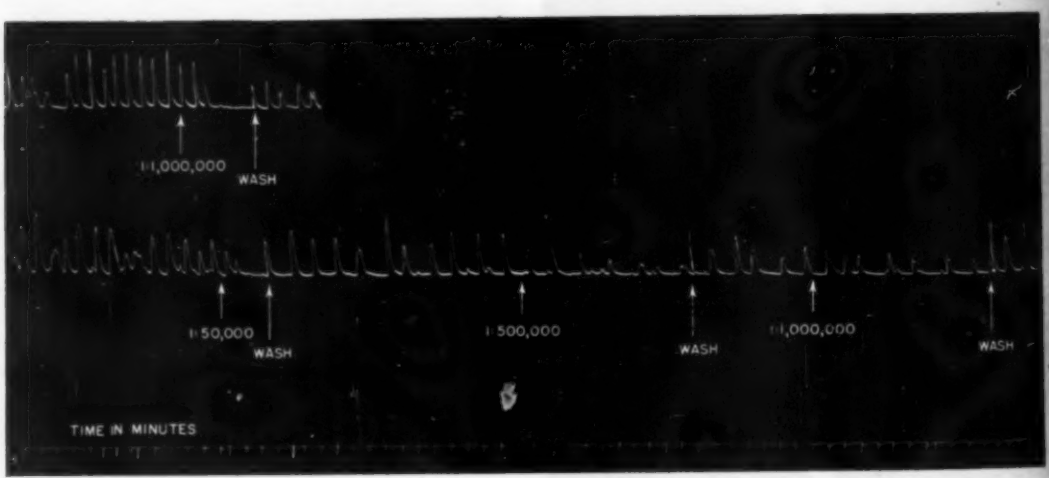
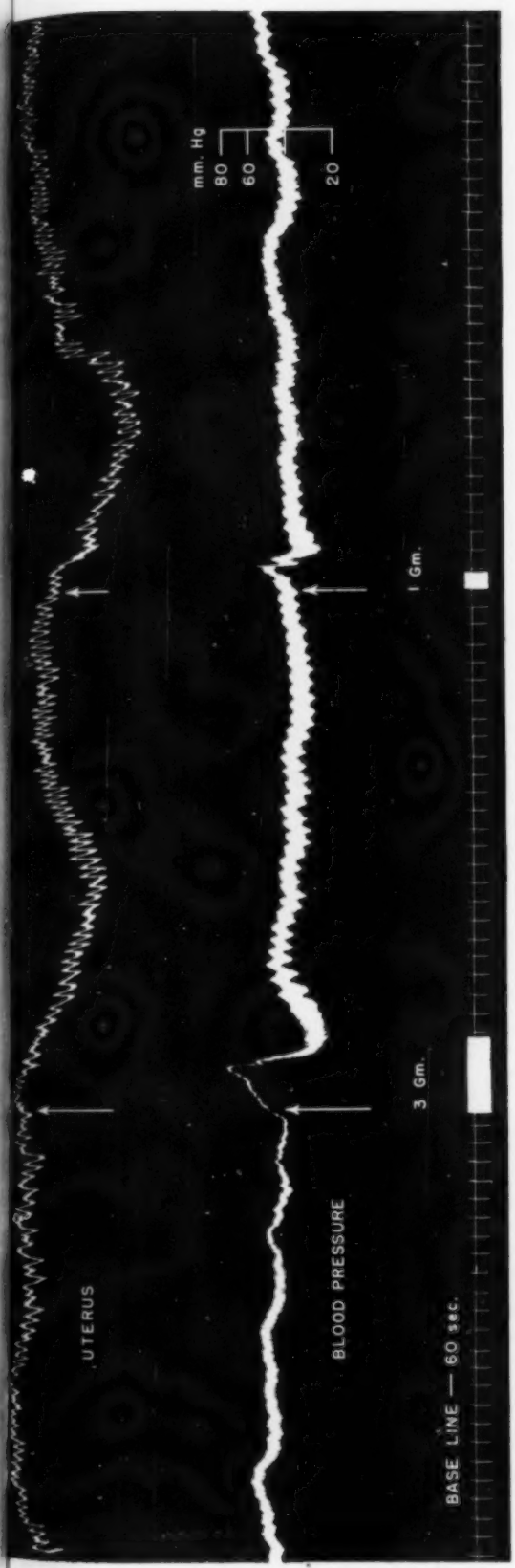


Fig. 4.—Effect on rat (anestrus), isolated uterus, right horn, *in vitro*. Drug: Piscidia, alcohol soluble portion of petroleum ether extract.



Petroleum Ether Extract.—A yellowish green resinous material which separated out from the petroleum ether solution on cooling was found to be significantly active on the uterus. A sufficient quantity was not available for an adequate chemical study. The material amounts to 0.0058% of the bark, is completely soluble in 95% alcohol and alkali, almost insoluble in water, and in its crude state melts between 78–81°. Danckwortt and Schütte (9) report a crystalline alcohol of the paraffin group ($C_{17}H_{36}O$, m. p. 79°) which they also obtained from the petroleum ether extract of this drug. In two *in vitro* experiments, significant inhibition of uterine activity was seen with a concentration of 1:10,000 expressed in terms of the dried drug.

Following the removal of the precipitate, a portion of the petroleum ether solution was distilled to a small volume and then evaporated spontaneously. During the course of the evaporation, a yellowish amorphous substance came down, similar to the precipitate obtained previously. This was not separated, however. The alcohol-soluble portion of the petroleum ether extract represented 0.3733% of the bark. Upon bioassay it was found to have a high order of activity on the uterus. In a series of four *in vitro* experiments, the threshold dose for inhibition of uterine activity was found to be 1:1,000,000 in terms of the dried drug. Larger concentrations showed proportionally more sedation (Fig. 4).

In vivo, filtered and buffered intravenous doses prepared by shaking 11 mg. of this fraction (representing 3 Gm. of drug) with saline solution usually produced marked drops in tonus with some decrease in rate and amplitude (Fig. 5).

Another portion of the petroleum ether extract, upon shaking repeatedly with 1% HCl, yielded no alkaloid to the acid that would give positive tests with the general reagents. Here again, the petroleum ether extract was evaporated and the residue picked up with 95% alcohol. During the course of the evaporation, more of the yellow amorphous material mentioned above was deposited but was not separated. The alcohol solution was again evaporated and picked up in a minimum of alcohol. Upon evaporation of the latter, there was obtained a dark brown viscous material, amounting to 0.123% of the bark. As might be expected, this fraction was of the same order of potency as the precipitate and the alcohol-soluble portion of the petroleum ether extract mentioned above.

In the opinion of the writers, the variance of potency in these three fractions of the petroleum ether extract may be due to the concentration of the yellow amorphous material, and not only to the variance in response of one uterus from another. Much more study is indicated upon the petroleum ether-soluble portion of this drug. It is shown later

Fig. 5.—Effect on cat, 1.65 Kg., *in vivo*. Drug (intravenous): Piscidia, alcohol soluble portion of petroleum ether extract, in saline.

in this paper that the fractions mentioned above have approximately the same order of toxicity against fish as did the principle which Hauschild (4) obtained in a similar manner.

Ether Extract.—Several fractions from this extract proved to be very active inhibitors of uterine contractions *in vitro*. The first of these was a light brown amorphous substance, representing 0.0064% of the bark which was deposited on cooling the ether solution. This residue was found to be effective in a dilution of 1:10,000 in terms of the drug or 1:156,000,000 in terms of yield of residue.

After the removal of the above material, the ether solution was reduced to a volume of 150 cc. whereupon a dark brown resinous material was deposited, amounting to 0.0835% of the bark. Its alcoholic solution when tested pharmacologically showed effective sedation in concentrations as small as 1:167,000 of the drug or 1:208,332,000 of the material itself.

The residual ether solution yielded a dark green semisolid which was extracted with a minimum of 95% alcohol. The alcohol-soluble portion represented 0.7% of the bark. This fraction, although much less potent than the ether fractions mentioned before, still is an active inhibitor of the contractions of the isolated rat uterus. In a series of several experiments using this material, definite sedation was shown using a concentration of 1:50,000 of the drug or 1:7,142,000 of the extract itself.

Chloroform Extract.—The residue from this extract was a dark viscid material amounting to 0.317% of the bark. Although quite active as a uterine depressant, this fraction is not of the same order of potency as those previously reported for petroleum ether. In the few *in vitro* experiments made, concentrations of 1:35,000 of the drug or 1:11,000,000 of the extract produced uterine sedation.

An attempt to further separate the active principle of this fraction was made employing partially the methods used by Freer and Clover (8) on the chloroform extract in determining the identity of piscidin. The chloroform residue was redissolved in 50 cc. of chloroform and allowed to stand for several days and then filtered free of any deposit. The filtrate was then evaporated to 25 cc., diluted with an equal volume of ether, and then extracted with a 33% solution of KOH. The chloroform-ether solution was repeatedly washed with water until the latter became colorless, then with a trace of acid, finally dried over anhydrous sodium sulfate, and the ether-chloroform distilled. The separation was carried no further than this point. A reddish brown, partially crystalline material was obtained amounting to 0.037% of the bark. This proved to be the carrier of the active principle of this fraction, since the effective dilution in terms of bark was approximately the same as that of the chloroform extract from which it was derived. In terms of the material itself, it proved to be effective *in vitro* in dilutions up to 1:280,000,000. Cushny (14), however, in testing crystalline piscidin obtained in this manner, found it to be inactive. It is not known, however, if uterine studies were made. Pittenger and E'We (17), on the other hand, found it an effective hypnotic in dogs.

Absolute Methanol and 70% Ethanol Extracts.—Although quite active when compared to other botanicals used for this purpose, these extracts were not of the same order of potency as the petroleum ether and ether fractions. Upon being tested *in vitro*, the methanol extract was found to be effective in a concentration of 1:10,000 of the drug or 1:500,000 of the extract itself. Concentrations of 1:5000 of the 70% ethanol extract showed but questionable sedation.

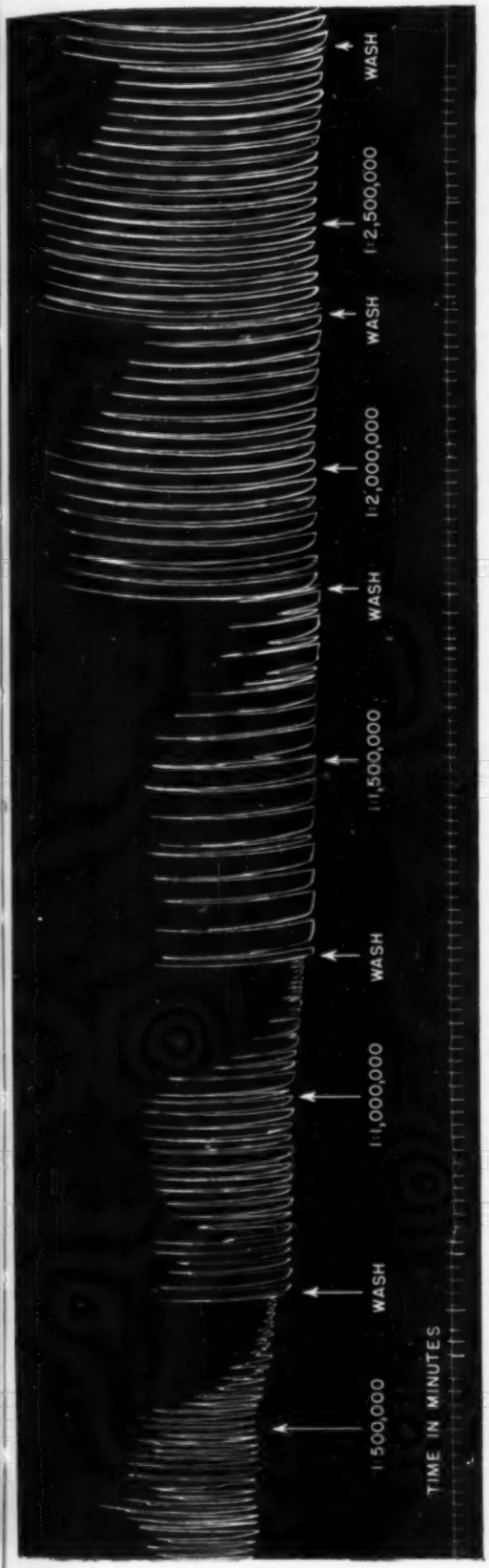
Determination of Optimum Solvent.—It has been shown that alcohol is by no means the optimum solvent for the uterine depressant principles of Piscidia. In order to determine the best single solvent for the active principles of the drug from a pharmaceutical standpoint, several of the other common solvents were tried on separate portions of commercial bark and the resultant dry extracts bioassayed. Table I illustrates the comparative merits of those employed.

TABLE I.—*Piscidia erythrina*. COMPARATIVE POTENCY *in Vitro* OF EXTRACTS OBTAINED WITH VARIOUS SOLVENTS

Solvent	Approx. Effective Dilution in Terms of Bark	Approx. Effective Dilution in Terms of Dry Extract	% of Drug Represented by Dry Extract	Method of Extraction
38.5% Ethanol[a]	1:20,000	1:265,000	7.53	Percolation
95% Ethanol	1:50,000	1:1,600,000	3.17	Soxhlet
60% Isopropanol	1:50,000	1:500,000	10.26	Percolation
Acetone	1:100,000	1:3,600,000	2.78	Soxhlet
Hot Water	1:111,000	1:488,000	22.73	Soxhlet
Chloroform	1:500,000	1:17,800,000	2.85	Soxhlet
Benzene	1:750,000	1:83,000,000	0.904	Soxhlet
Petroleum Ether[b]	1:1,000,000	1:260,000,000	0.373	Soxhlet

[a] Fluidextract.
[b] Alcohol-soluble portion.

Determination of Most Active Part of Plant.—Due to the contradictions in the literature as to the part used, and because past investigations have been limited to the stem and root barks, it was decided to investigate the comparative potencies of several parts of the plant. Having access to stands of the material in Florida and Jamaica, various parts of the



plant were collected for us which we submitted to bioassay. Portions of the various parts were refluxed with 95% alcohol containing calcium carbonate for over three hours and filtered hot. The residues were washed with hot alcohol and the washings added to the filtrates. Although a limited number of experiments were conducted, and different uteri varied in response, sufficient data were collected to demonstrate the comparative potency of the parts tested and to indicate that all these parts bear a potent principle depressant to the uterus *in vitro*. The part most potent appears to be the root bark which is effective in dilutions of from 1:1,000,000 to 1:3,000,000 (Fig. 6).

Table II illustrates these findings, expressed in average threshold dilutions in terms of part used.

TABLE II.—*Piscidia erythrina*. COMPARATIVE POTENCIES *in Vitro* OF VARIOUS PARTS OF THE PLANT

Part Used	Source	State	Effective Dilution
Leaves	Florida	Dried	1:100,000
	Jamaica	Dried	1:100,000
Young whole limbs	Jamaica	Dried	1:100,000
Young stem bark	Florida	Fresh	1:50,000
	Florida	Dried	1:200,000
Older stem bark	Jamaica	Dried	1:500,000
Young stem wood	Florida	Fresh	1:1,000,000
	Florida	Dried	1:500,000
Root wood	Florida	Fresh	1:500,000
	Florida	Dried	1:500,000
Root bark	Florida	Fresh	1:3,000,000
	Florida	Dried	1:1,000,000

On standing, crystalline material was deposited in the alcoholic extract of most of the above. This was separated and recrystallized, and a yield of white granular crystals, melting at 188°, was obtained amounting to 0.13% of the part used in the cases of the tree and root barks. The crystals had a sweet taste, were soluble in water and dilute alcohol, slightly soluble in 95% alcohol and absolute methanol, and insoluble in petroleum ether, ether, chloroform, acetone, and benzene. The principle reduced Fehling's solution only after hydrolysis with diluted hydrochloric acid at 100°. Upon bioassay, the principle was found to be inactive upon uterine tissue *in vitro* and *in vivo*, and no further chemical study was conducted.

Comparison of Uterine Sedative Effect (*in Vitro*).—It seemed a point of interest to us that a comparison be made of the inhibitory property of various standard preparations on uterine tissue and the sedative effect of certain Piscidia fractions. The experiments from which Table III was compiled were made in our laboratory and represent the activity of the various preparations as we have found

Fig. 6.—Effect on rat, 149 Gm. (anestrus), isolated uterus, right horn, *in vitro*. Drug: Piscidia, root bark extraction of dried drug, 95% alcohol.

TABLE III.—COMPARISON OF SEDATIVE DOSAGE OF PISCIDIA AND VARIOUS OTHER SMOOTH MUSCLE DEPRESSANTS (RAT UTERUS—*in Vitro*)

		Effective Sedative Dose (in Terms of Dried Drug)	Index of Potency (Viburnum Taken as 1)	Effective Sedative Dose (in Terms of Dried Extract)
1.	Fluidextract *Viburnum prunifolium*	1:1000	1	.....
2.	Fluidextract *Piscidia erythrina*	1:20,000	20	.....
3.	Ethanol (95%) Extract, Piscidia	1:50,000	50	1:1,600,000
4.	Acetone Extract, Piscidia	1:100,000	100	1:3,600,000
5.	Benzene Extract, Piscidia	1:750,000	750	1:83,000,000
6.	Ethanol-soluble portion of Petroleum Ether Extract, Piscidia	1:1,000,000	1000	1:260,000,000
7.	Papaverine Hydrochloride	1:1,000,000	1000	.....
8.	Ethanol Soxhlet of Root Bark, Piscidia	1:3,000,000	3000	.....

them. It will be noted that certain fractions of Piscidia compare favorably in action with Papaverine Hydrochloride which has been proposed as a standard (29, 30) for bioassay of uterine sedatives.

Toxicity.—In view of its history as a fish poison and hypnotic, a limited number of experiments were performed on laboratory animals and gold fish to ascertain the relative toxicity of Piscidia and some of its principles. These toxicity studies were not intended to be complete, but to serve as sighting experiments for further work. The fluidextract given orally *via* catheter to a group of twenty rats proved nontoxic at the dosage level of 20 Gm. of the drug per Kg. Symptoms such as grogginess and drowsiness could be explained partially by the hypnotic effect of the drug and the alcohol contained in the fluidextract. Control animals given the same volume of alcohol showed the same symptoms but in somewhat lesser degrees. All animals recovered within five to seven hours. Another series of rats was given a 60% isopropyl alcohol dried extract of Piscidia dispersed in water. Two out of a group of seven receiving 10,000 mg. per Kg. orally of the extract, equivalent to 106 Gm. of the dried bark, died within one hour, while the remaining five recovered. A group of ten rats receiving 7500 mg. per Kg. of the extract, equivalent to 80 Gm. of the dried bark, recovered within eight hours.

Hauschild (4) found the alcohol-soluble principle of the petroleum ether extract to be toxic to fish in dilutions of 1:80,000,000. In a series of tests employing thirty gold fish, we found the principle to be toxic in from 1:5,000,000 to 1:30,000,000. The lower toxic concentration may be explained in that larger fish were employed in our experiments.

SUMMARY

1. The present bark of commerce of Piscidia represents the stem bark, and not the root bark as reported by earlier investigators.

2. Preparations of Piscidia were found to be exceedingly potent uterine depressants *in vitro* and *in vivo* on various laboratory animals.

3. The drug was submitted to chemical separation in order to trace the depressant compounds. Such substances were found in the resin and the extracts of alcohol, petroleum ether, ether, and chloroform.

4. Petroleum ether is the optimum single solvent for the depressant principle.

5. All parts of the plant contain the depressant principle, the root bark being the most potent.

6. Certain fractions of the drug have the same order of uterine depression *in vitro* as Papaverine Hydrochloride and exceed any of the known botanicals used for this purpose in depressant action.

7. Acute studies indicate that Piscidia has a low relative toxicity when fed orally to rats. The work of Hauschild on the fish poisoning principle has been partially confirmed.

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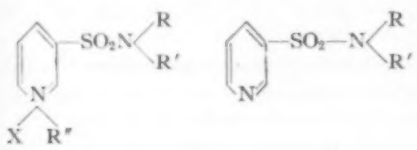
Quaternary Salts of N′-Substituted Pyridine-3-Sulfonamides*

By M. F. ZIENTY†

Methods for the preparation of several new N′-substituted pyridine-3-sulfonamides are described. From these eighteen new quaternary salts, several which possess germicidal activity were derived.

DOMAGK'S demonstration (1) of the bactericidal properties of quaternary salts in which the nitrogen bears a long chain aliphatic substituent stimulated a number of investigations in this field (2–5).

A previous report from this laboratory (6) indicated that alkylpyridinium and alkyl picolinium halide salts possess conspicuous germicidal activity. The present communication describes the preparation and germicidal activity of a new class of quaternary salts having the general structure (I).



I II

R and R′ = alkyl radicals H, C_2H_5, C_9H_{17}, $C_{12}H_{25}$, $C_{14}H_{29}$, or $C_{16}H_{33}$
R″ = CH_3, C_7H_7, C_3H_5, $C_{12}H_{25}$, or $C_{16}H_{33}$
X = Cl, Br, I, or SO_4CH_3

Compounds of this class (I) were chosen in order to determine the efficacy of quaternary ammonium salts containing a long chain aliphatic substituent attached to the basic heterocyclic ring by other than a carbon-to-carbon bond or direct connection to the quaternary nitrogen.

Sulfonation of pyridine yielded pyridine-3-sulfonic acid (7, 8). After conversion to the corresponding sulfonyl chloride (7), the pyridine-3-sulfonamides (II) listed in Table I were prepared in yields of 63–77%.

The eighteen quaternary salts shown in Table II were obtained from II by one of the several methods described previously (6, 9). As indicated in this table, the germicidal activity of a number of these compounds compares favorably with the values reported for benzalkonium chloride (Zephiran) (10).

TABLE I.—N′-SUBSTITUTED PYRIDINE-3-SULFONAMIDES

Pyridine-3-sulfonamide	M. p., °C.	Formula	Nitrogen, % Calcd.	Nitrogen, % Found
N′-Dodecyl	67–68	$C_{17}H_{30}O_2N_2S$	8.61	8.75
N′-Tetradecyl	79–80	$C_{19}H_{34}O_2N_2S$	7.91	7.71
N′-Hexadecyl	78–79	$C_{21}H_{38}O_2N_2S$	7.32	7.07
N′-Di-2-ethylhexyl	Oil[a]	$C_{21}H_{38}O_2N_2S$	7.31	7.51
N′-Diethyl	46–47[b]	$C_9H_{14}O_2N_2S$	..	..

[a] B. p. 253–255°/16 mm.
[b] Machek, *Monatsh.*, **72**, 87(1939); reported m. p. 49–50°.

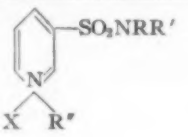
EXPERIMENTAL[1]

Pyridine-3-sulfonic acid.—To a mixture of 4.2 Gm. of mercuric sulfate and 700 Gm. of 30% oleum was added, cautiously, 120 Gm. (1.2 moles) of pyridine. The solution was heated under reflux at 300° (bath temperature) for eight hours. On cool-

* Received Oct. 3, 1947, from the Research Laboratories of The Upjohn Company, Kalamazoo, Mich.
† The author wishes to thank Dr. H. G. Kolloff for his valuable suggestions and interest in this work, and the Bacteriology Research Department of The Upjohn Company for the bacteriological data.

[1] All melting points are uncorrected. Microanalyses by Messrs. H. Emerson and W. Struck.

TABLE II.—QUATERNARY SALTS OF N-SUBSTITUTED PYRIDINE-3-SULFONAMIDES



R	R′	R″	X	M. p., °C.	Formula	Halogen, % Calcd.	Halogen, % Found	Activity [c]
$C_{12}H_{25}$	H	CH_3	I	96–97	$C_{19}H_{33}O_2N_2SI$	27.13	27.03	1–15,000
$C_{14}H_{29}$	H	CH_3	I	198–199	$C_{20}H_{37}O_2N_2SI$	25.60	26.91	1–20,000
$C_{16}H_{33}$	H	CH_3	I	165–166	$C_{22}H_{41}O_2N_2SI$	24.24	24.41	1–10,000
C_8H_{17}[a]	C_8H_{17}[a]	CH_3	I	144–145	$C_{22}H_{41}O_2N_2SI$	24.24	24.02	1–35,000
$C_{12}H_{25}$	H	C_7H_7	Cl	157–158	$C_{24}H_{37}O_2N_2SCl$	7.74	7.71	1–30,000
$C_{14}H_{29}$	H	C_7H_7	Cl	160–161	$C_{26}H_{41}O_2N_2SCl$	7.36	7.16	1–10,000
$C_{16}H_{33}$	H	C_7H_7	Cl	160–161	$C_{28}H_{45}O_2N_2SCl$	6.89	6.86	1–25,000
C_8H_{17}[a]	C_8H_{17}[a]	C_7H_7	Cl	Oil	$C_{23}H_{43}O_2N_2SCl$	6.89	6.82	1–20,000
$C_{12}H_{25}$	H	C_3H_5	Br	102–103	$C_{25}H_{35}O_2N_2SBr$	17.89	17.76	1–10,000
$C_{14}H_{29}$	H	C_3H_5	Br	109–110	$C_{22}H_{39}O_2N_2SBr$	16.84	16.95	1–10,000
$C_{16}H_{33}$	H	C_3H_5	Br	105–106	$C_{24}H_{43}O_2N_2SBr$	15.90	15.70	1–25,000
C_8H_{17}[a]	C_8H_{17}[a]	C_3H_5	Br	74–75	$C_{24}H_{43}O_2N_2SBr$	15.90	15.88	1–25,000
$C_{12}H_{25}$	H	CH_3	SO_4CH_3	Oil	$C_{19}H_{36}O_6N_2S_2$	(6.19)[b]	(6.21)[b]	1–1,000
$C_{14}H_{29}$	H	CH_3	SO_4CH_3	110–111	$C_{21}H_{40}O_6N_2S_2$	(6.25)[b]	(6.14)[b]	1–1,000
$C_{16}H_{33}$	H	CH_3	SO_4CH_3	104–105	$C_{23}H_{44}O_6N_2S_2$	(5.51)[b]	(5.77)[b]	1–1,000
C_8H_{17}[a]	C_8H_{17}[a]	CH_3	SO_4CH_3	Oil	$C_{23}H_{44}O_6N_2S_2$	(5.51)[b]	(5.72)[b]	1–10,000
C_2H_5	C_2H_5	$C_{12}H_{25}$	Br	95–97	$C_{21}H_{39}O_2N_2SBr$	17.31	17.56	1–1,000
C_2H_5	C_2H_5	$C_{16}H_{33}$	Br	77–78	$C_{23}H_{47}O_2N_2SBr$	15.41	15.74	1–1,000

[a] 2-Ethylhexyl.
[b] Nitrogen, %.
[c] Determined by the F.D.A. method, using *Staphylococcus aureus* at 37°, described in Circular 198 of the U. S. Department of Agriculture.

ing, the reaction mixture was poured into 1 L. of cracked ice and water. The solution was neutralized with solid barium carbonate and the precipitated barium sulfate removed by suction filtration. The filter cake was washed well with hot water to remove all the sulfonic acid. The filtrate and washings were combined and evaporated nearly to dryness. Treatment of the residue with concentrated sulfuric acid followed by filtration to remove barium sulfate and concentration of the filtrate yielded 131 Gm. (55%) of colorless needles, m. p. 365–366°.

Pyridine-3-sulfonyl chloride.—Ten grams (0.078 mole) of pyridine-3-sulfonic acid was triturated in a mortar with 13.2 Gm. of phosphorus pentachloride, transferred to a ground-glass jointed flask and heated under reflux at 160–165° until the evolution of hydrogen chloride has ceased (two to three hours). The flask was fitted with an inclined condenser and the phosphorus oxychloride removed under reduced pressure. The residual yellowish oil was the crude pyridine-3-sulfonyl chloride which was used in subsequent reactions without further purification.

Pyridine-3-N′-alkylsulfonamides.—The N′-alkylsulfonamides listed in Table I were prepared by the procedure used to synthesize pyridine-3-N′-hexadecylsulfonamide.

Pyridine-3-N′-hexadecylsulfonamide.—A solution of pyridine-3-sulfonyl chloride in 50 cc. of dry benzene, prepared from 10 Gm. (0.078 mole) of pyridine-3-sulfonic acid, was well cooled in ice and treated with a solution of 30 Gm. (0.12 mole) of hexadecylamine in 100 cc. of dry benzene. The copious precipitate of amine hydrochloride formed was removed by suction filtration and the filtrate evaporated to dryness. The solid residue was crystallized from a mixture of benzene and petroleum ether (90–100°), and yielded 15 Gm. (63%) of colorless crystals, m. p. 78–79°.

Anal.—Calcd. for $C_{21}H_{38}N_2O_2S$: N, 7.32. Found: N, 7.07.

Pyridine-3-N′-hexadecylsulfonamide Methiodide.—A solution of 1.25 Gm. (0.0033 mole) of pyridine-3-N′-hexadecylsulfonamide and 0.60 Gm. (0.0042 mole) of methyl iodide in 15 cc. of absolute alcohol, was refluxed for six hours. The solvent was removed by evaporation and the yellow solid dissolved in ethyl acetate. On cooling, 1.5 Gm. (78%) of a light yellow amorphous solid was obtained, m. p. 165–166°.

Anal.—Calcd. for $C_{22}H_{40}N_2O_2SI$: I, 24.28. Found: I, 24.41.

Pyridine-3-N′-hexadecylsulfonamide Benzylchloride.—A mixture of 1.5 Gm. (0.004 mole) of pyridine-3-N′-hexadecylsulfonamide and 0.49 Gm. (0.004 mole) of benzyl chloride was heated in an oil bath at 110° for two hours. On cooling, a solid crystalline mass was obtained which was recrystallized from ethyl acetate to yield 1.8 Gm. (90%) of lustrous plates, m. p. 160–161°.

Anal.—Calcd. for $C_{29}H_{45}N_2O_2SCl$: Cl, 6.97. Found: Cl, 6.86.

Pyridine-3-N′-hexadecylsulfonamide Allylobromide.—One gram (0.0026 mole) of pyridine-3-N′-hexadecylsulfonamide and 0.35 Gm. (0.003 mole) of allyl bromide was heated under reflux for ten minutes. The heating was interrupted, the flask stoppered and allowed to stand at room temperature overnight. The crystalline mass was dissolved in

acetone and skelly-solv "B" added until the solution became slightly turbid. On standing, 1 Gm. (80%) of nearly colorless plates was obtained, m. p. 105–106°.

Anal.—Calcd. for $C_{24}H_{43}N_2O_2SBr$: Br, 15.90. Found: Br, 15.70.

Pyridine-3-N′-hexadecylsulfonamide Methosulfate.—A mixture of 2 Gm. (0.0052 mole) of pyridine-3-N′-hexadecylsulfonamide and 0.75 Gm. (0.006 mole) of dimethyl sulfate was heated on a steam cone for three hours. The unreacted dimethyl sulfate was removed by washing with petroleum ether. The semisolid was dissolved in ethyl acetate, decolorized, and filtered. Colorless plates were obtained; yield 2.1 Gm. (80%), m. p. 104–105°.

Anal.—Calcd. for $C_{23}H_{41}N_2O_6S_2$: N, 5.51. Found: N, 5.77.

Pyridine-3-N′-diethylsulfonamide Laurylobromide.—Ten grams (0.047 mole) of pyridine-3-N′-diethylsulfonamide was heated with 11.7 Gm. (0.047 mole) of lauryl bromide at 120° for two hours. Recrystallization of the solid from a mixture of petroleum ether and dry ether yielded 18 Gm. (83%) of colorless crystals, m. p. 95–97°.

Anal.—Calcd. for $C_{23}H_{43}N_2O_2SBr$: Br, 17.31. Found: Br, 17.56.

SUMMARY

Details are given for the preparation of several new N′-substituted pyridine-3-sulfonamides and, from these, eighteen new quaternary salts. The germicidal activity of several of the new quaternary salts has been shown to compare favorably with other established germicides.

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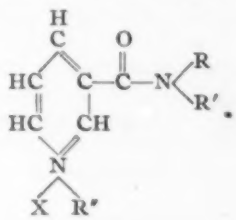
Quaternary Salts of Pyridine-3-Carboxamides*

By M. F. ZIENTY†

The preparation of twenty-seven salts of nicotinamide, N′-N′-diethylnicotinamide and 4-(nicotinyl) morpholine, is reported. Each of these compounds were found to be active germicides.

THE high germicidal activity of a number of pyridinium, 2-picolinium and 4-picolinium salts (1) suggested that other quaternary salts of substituted pyridines be prepared and evaluated.

A survey of the literature disclosed that several quaternary salts of nicotinamide (2) and the methiodide and methochloride of nikethamide (3), but no quaternary salts of 4-(nicotinyl)-morpholine, had been prepared. However, none of these had been tested for germicidal activity. Therefore it seemed desirable to prepare a series of quaternary salts of these amides and explore the possibilities of this class of compounds having the general structure (I).



I

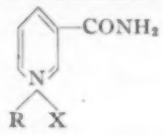
R and R′ = H, C_6H_5 or
$-CH_2-CH_2-O-CH_2-CH_2-$
R″ = $C_{12}H_{25}$, $C_{14}H_{29}$, $C_{16}H_{33}$, or $C_{18}H_{37}$
X = Cl, Br, or I

The quaternary salts of nicotinamide were prepared by heating equivalent amounts of amide with an alkyl halide at 150–155° for eight hours.

The reaction between 4-(nicotinyl)-morpholine or nikethamide and an alkyl halide, without a solvent, gave only dark-

* Received Oct. 3, 1947, from the Research Laboratories of The Upjohn Company, Kalamazoo, Mich.
† The author wishes to thank Dr. H. G. Kolloff for his advice and suggestions in this work, and the Bacteriology Research Department of The Upjohn Company for the bacteriological data.

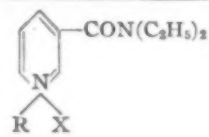
TABLE I.—ALKYL-3-CARBAMYLPYRIDINIUM HALIDES



R	X	M. p., °C.	Formula	Nitrogen, % Caled.	Found	Activity
$C_{12}H_{25}$	Cl	237–239	$C_{18}H_{31}N_2OCl$	8.58	8.55	1–5,000
$C_{14}H_{29}$	Cl	246–247	$C_{20}H_{35}N_2OCl$	(9.88)[a]	(9.02)[a]	1–15,000
$C_{16}H_{33}$	Cl	245–246[b]	$C_{22}H_{39}N_2OCl$	7.33	7.56	1–15,000
$C_{12}H_{25}$	Br	229–230	$C_{18}H_{31}N_2OBr$	7.54	7.42	1–5,000
$C_{14}H_{29}$	Br	229–230	$C_{20}H_{35}N_2OBr$	7.02	6.98	1–15,000
$C_{16}H_{33}$	Br	225–226	$C_{22}H_{39}N_2OBr$	6.55	6.49	1–15,000
$C_{18}H_{37}$	Br	224–225	$C_{24}H_{43}N_2OBr$	4.50	4.79	1–2,000
$C_{12}H_{25}$	I	177–178	$C_{18}H_{31}N_2OI$	(30.30)[a]	(29.80)[a]	1–10,000
$C_{14}H_{29}$	I	176–178	$C_{20}H_{35}N_2OI$	(28.42)[a]	(29.62)[a]	1–5,000
$C_{16}H_{33}$	I	175–177	$C_{22}H_{39}N_2OI$	5.90	5.52	1–5,000
$C_{18}H_{37}$	I	173–175	$C_{24}H_{43}N_2OI$	5.57	5.48	1–2,000

[a] Halogen, %.
[b] Karrer and Stare, *Helv. Chim. Acta*, **20**, 423(1937); reported m. p. 235°.

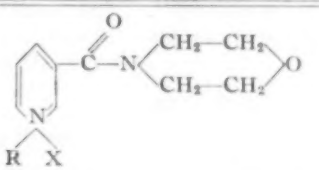
TABLE II.—ALKYL-3-DIETHYLCARBAMYLPYRIDINIUM HALIDES



R	X	M. p., °C.	Formula	Nitrogen, % Caled.	Found	Activity
$C_{12}H_{25}$	Br	Oil	$C_{22}H_{39}N_2OBr$	6.55	6.79	1–5,000
$C_{14}H_{29}$	Br	57–58	$C_{24}H_{43}N_2OBr$	6.15	6.28	1–10,000
$C_{16}H_{33}$	Br	63–64	$C_{26}H_{47}N_2OBr$	5.79	5.88	1–10,000
$C_{18}H_{37}$	Br	68–69	$C_{28}H_{51}N_2OBr$	(15.63)[a]	(14.34)[a]	1–5,000
$C_{12}H_{25}$	I	Oil	$C_{22}H_{39}N_2OI$	5.90	5.84	1–5,000
$C_{14}H_{29}$	I	Oil	$C_{24}H_{43}N_2OI$	5.57	5.73	1–5,000
$C_{16}H_{33}$	I	52–55	$C_{26}H_{47}N_2OI$	5.28	5.06	1–10,000
$C_{18}H_{37}$	I	62–63	$C_{28}H_{51}N_2OI$	(22.79)[a]	(23.05)[a]	1–5,000

[a] Halogen, %.

TABLE III.—QUATERNARY SALTS OF 4-(NICOTINYL)-MORPHOLINE



R	X	M. p., °C.	Formula	Nitrogen, % Caled.	Found	Activity
$C_{12}H_{25}$	Br	54–55	$C_{22}H_{37}N_2O_2Br$	6.34	6.55	1–2,000
$C_{14}H_{29}$	Br	83–85	$C_{24}H_{41}N_2O_2Br$	5.97	6.14	1–10,000
$C_{16}H_{33}$	Br	74–75	$C_{26}H_{45}N_2O_2Br$	5.63	5.82	1–10,000
$C_{18}H_{37}$	Br	78–79	$C_{28}H_{49}N_2O_2Br$	5.33	5.05	1–2,000
$C_{12}H_{25}$	I	144–145	$C_{22}H_{37}N_2O_2I$	5.74	5.90	1–2,000
$C_{14}H_{29}$	I	145–146	$C_{24}H_{41}N_2O_2I$	5.42	5.55	1–5,000
$C_{16}H_{33}$	I	152–153	$C_{26}H_{45}N_2O_2I$	5.30	5.39	1–2,000
$C_{18}H_{37}$	I	153–154	$C_{28}H_{49}N_2O_2I$	(22.20)[a]	(22.52)[a]	1–2,000

[a] Halogen, %.

colored tars. However, if the reactants were dissolved in dry xylene and refluxed for eight hours, colorless, crystalline products were obtained. Repeated attempts to react either 4-(nicotinyl)-morpholine or nikethamide with alkyl chlorides failed. The unreacted starting materials were always recovered. Nicotinamide used in this work was the U. S. P. XII product, available commercially, while the nikethamide and 4-(nicotinyl)-morpholine were prepared, essentially, according to directions in the literature (4).

Germicidal tests were made according to the F.D.A. method using *Staphylococcus aureus* at 37° (5).

The compounds were active as germicides. Without exception, the salts of the amides were less active than the corresponding pyridinium or picolinium compounds (1). The most active compounds were the quaternary salts of nicotinamide, those of nikethamide next, and those of 4-(nicotinyl)-morpholine the least active. The solubilities of these compounds, in aqueous solutions, were in the reverse order.

EXPERIMENTAL[1]

4-(Nicotinyl)-morpholine.—A mixture of 92 Gm. (1.05 moles) of morpholine and 123 Gm. (1 mole) of nicotinic acid was cooled in ice, cautiously treated with 77 Gm. (0.5 mole) of phosphorous oxychloride, and the solution heated on a steam bath, under reflux, for ten hours. The resulting semisolid reaction mixture was dissolved in 400 cc. of water and the solution saturated with solid potassium carbonate. The upper oily layer was separated and dried under reduced pressure at the temperature of the steam bath. The nearly solid slurry was treated with 400 cc. of dry benzene and the inorganic salts removed by suction filtration. Removal of the benzene under reduced pressure gave an oil which boiled at 196–200°/10 mm.,[2] yielding 69 Gm. (35%) of a heavy yellow oil.

N′,N′-Diethylnicotinamide.—A three-liter, three-necked flask, fitted with a stirrer, reflux condenser, and dropping funnel was charged with 492 Gm. (4 moles) of nicotinic acid. The stirrer was started and 308 Gm. (4.2 moles) of diethylamine allowed to run into the flask, with external cooling. Then 608 Gm. of phosphorous oxychloride was slowly dropped into the mixture. The stirring was interrupted and the dark solution heated on a steam bath overnight. The thick, sirupy liquid was poured into a mixture of cracked ice and sodium hydroxide solution (500 Gm. sodium hydroxide in 500 cc. of water). The upper layer was separated and the lower aqueous layer extracted with four 200-cc. portions of benzene. The organic layer and extracts were combined and a small amount of water separated. Removal of the benzene under reduced pressure gave a dark oil which was distilled to yield 498 Gm. (70%) of nearly colorless product; b. p. 163–168°/11 mm.

Lauryl-3-carbamylpyridinium Bromide.—A mixture of 12.2 Gm. (0.1 mole) of nicotinamide and 25 Gm. (0.1 mole) of lauryl bromide was heated in a metal bath, so that an internal temperature of 150–155° was maintained, for eight hours. The mixture was cooled and the solid dissolved in hot ethanol, decolorized with Darco and filtered. On cooling, 26 Gm. (70%) of yellowish crystals were obtained. Several crystallizations yielded colorless material, m. p. 227–228°.

Anal.—Calcd. for $C_{18}H_{31}N_2OBr$: N, 7.54. Found: N, 7.22.

Dodecyl-4-(nicotinyl)-morpholine Iodide.—A solution of 10 Gm. (0.05 mole) 4-(nicotinyl)-morpholine and 14.8 Gm. (0.05 mole) of dodecyl iodide in 40 cc. of dry xylene was heated under reflux for eight hours. The solvent was removed and the semisolid, obtained on cooling, rubbed under ethyl acetate until crystallization took place. Two crystallizations from ethyl acetate yielded 11 Gm. (50%) of lemon-colored crystals, m. p. 144–145°.

Anal.—Calcd. for $C_{22}H_{37}N_2O_2I$: N, 5.74. Found: N, 5.90.

Hexadecyl-3-diethylcarbamylpyridinium Bromide.—A solution of 10 Gm. (0.056 mole) of nikethamide and 17 Gm. (0.056 mole) of hexadecyl bromide in 40 cc. of dry xylene was refluxed for eight hours. The xylene was removed and the semisolid mass, obtained on cooling, dissolved in ethyl acetate and a small amount of ethanol. On cooling, 8 Gm. (30%) of colorless crystals were obtained; m. p. 63–64°.

Anal.—Calcd. for $C_{26}H_{47}N_2OBr$: N, 5.79. Found: N, 5.88.

SUMMARY

A series of twenty-seven salts of nicotinamide, N′,N′-diethylnicotinamide and 4-(nicotinyl) morpholine has been prepared.

The compounds are active germicides.

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[1] All melting points are uncorrected. Microanalyses by Messrs. H. Emerson and W. Struck and Misses Celia Triemstra and Barbara Fausnaugh.

[2] French Patent 824,042(1938); *C. A.*, **32**, 4285(1938); reported b. p. 210°/15 mm.

Synthesis of Tetraiodinated Congo Red*†‡

By CHARLES F. MARTIN§ and HAAKON BANG‖

3,3′,5,5′-Tetraiodobenzidine and tetraiodinated Congo red were prepared. The compounds were analyzed for their iodine content and their solubilities were determined. Pharmacological and radiological studies of tetraiodinated Congo red have thus far indicated that it is nontoxic, but that it is not concentrated in amyloid tissue in sufficient quantity to produce any opacity with X-ray. Further tests of this type are anticipated.

THIS report is based on a part of the work undertaken by the authors in the search for new iodinated compounds which might find use as radiographic opaque agents. Pharmacological and radiological studies have indicated, thus far, that tetraiodinated Congo red is no more toxic than Congo red, but that, after administration, the concentration of it in amyloid tissue is not great enough to cast a shadow on the X-ray plate (1). Further pharmacological and radiological studies of the compound are anticipated.

EXPERIMENTAL

3,3′,5,5′-Tetraiodobenzidine.—Nineteen and two-tenth grams of benzidine dihydrochloride was dissolved, by the aid of heat and constant mechanical stirring, in 1200 cc. of distilled water containing 184 cc. of concentrated hydrochloric acid. When solution was complete, stirring was continued and the temperature was adjusted to 80°. At this time, 1500 cc. of a solution containing 75 Gm. of iodine and 150 Gm. of potassium iodide was added in 100-cc. portions during a period of about thirty minutes. Stirring was continued and the temperature was maintained at 80° for two hours after the last of the iodine solution had been added.

The dark red solution was removed from the heat and allowed to cool to room temperature. The crystals, thus obtained, were collected, dried in air, and washed with three 200-cc. portions of cold carbon tetrachloride. The product was then dried in air and analyzed for its iodine content by the method used by Klemme and Hunter (2).

Anal.—Calcd. for $C_{12}H_8I_4N_2$: I, 73.8%. Found: I, 73.3% and 73.1%.

The product is purple-colored needles which will not melt when heated to 300°. It is insoluble in water, carbon tetrachloride, chloroform, petroleum ether, benzene and acetic acid. It is soluble in ethanol, methanol, acetone and ether. The yield was 98%.

Tetraiodinated Congo Red.—Forty and eight tenths grams of finely powdered 3,3′,5,5′-tetraiodobenzidine was suspended in 300 cc. of 10% hydrochloric acid. This mixture was cooled in an ice-salt bath to 0° with constant stirring. A solution containing 12.6 Gm. of sodium nitrite in 15 cc. of cold distilled water was added in small portions to the mixture. Stirring was continued at the lowered temperature for thirty minutes after all of the sodium nitrite solution had been added.

The cold mixture containing the tetrazotized compound was poured, in small portions, into a stirred solution containing 30.6 Gm. of sodium naphthionate and 90 Gm. of sodium carbonate in 1200 cc. of cold distilled water. When effervescence had subsided, the dark-red, alkaline solution was heated to boiling and allowed to stand overnight. The solution was filtered and the filtrate was evaporated to dryness on a water bath. The residue was powdered in a mortar and extracted with a liter of absolute methanol the first time, and then with three 400-cc. portions of the same solvent.

The methanol extracts were combined, filtered, and evaporated to dryness on a water bath. The residue was dried in an oven at 100°, powdered, and extracted once with 1200 cc. of absolute ethanol.

The ethanol extract was filtered and evaporated to dryness. The residue was powdered and then washed with a solution containing three parts of ether and one part of absolute ethanol. The dye was dried in an oven at 100° and then analyzed for its iodine content by the method used before.

Anal.—Calcd. for $C_{32}H_{18}I_4N_6Na_2O_6S_2$: I, 42.3%. Found: I, 42.8% and 42.7%.

The dye is a dark red powder, insoluble in ether, chloroform, acetone, petroleum ether and benzene. It is soluble in ethanol and methanol. One gram is soluble in about 2.4 Gm. of water. The yield was 51.8%.

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† This paper is based on a portion of a thesis to be submitted to the Graduate Studies Committee of the State College of Washington by Charles F. Martin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
‡ This investigation was supported by a grant from Lederle Laboratories, Inc., Pearl River, New York.
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Pharmacognostical Studies of Punarnavā

Stem and Leaf Characteristics of *Boerhaavia diffusa* Linn. and *Trianthema Portulacastrum* Linn.*

By S. PRASAD†

Punarnavā has been employed in India from earliest times as a specific against edema, beriberi, anemia, and heart diseases. The pharmacognostical characteristics of this drug are described and illustrated.

THERE exists some confusion with regard to the correct identity of the drug, Punarnavā, which has been employed in the Ayurvedic system of medicine as a specific against edema or dropsical conditions, beriberi, anemia, heart diseases, and also as a rejuvenant. Two varieties of this drug, namely, "Shwet Punarnavā" or white-flowered variety and "Rakta Punarnavā" or red-flowered variety, have been mentioned in the Ayurved. The plants corresponding to these have been identified as *Trianthema Portulacastrum* Linn. (*T. monogyna* Linn.) and *Boerhaavia diffusa* Linn. (*B. repens* Linn.), respectively [*cf.* Chakravarty (1)]. Sircar (2), however, is of the opinion that *Boerhaavia diffusa* which has been described to be Punarnavā by previous workers (3–6) is a different drug, "Kathillaka" by name, having similar therapeutic properties but being a little less potent than the real Punarnavā. According to him, *Trianthema Portulacastrum* is in fact Punarnavā, inasmuch as this plant possesses two kinds of flowers, one white and the other slightly red or pinkish; the white-flowered type thus represents "Shwet Punarnavā" and the other type as "Rakta Punarnavā."

The chemical and pharmacological studies of the above-mentioned species have been made by many investigators. Ghoshal (7) isolated from *Boerhaavia diffusa* an alkaloid, an oily mass of the nature of fat, sulfates and chlorides, and traces of nitrates and chlorates. Chopra, *et al.* (8) obtained sufficient amount of potassium nitrate and also an alkaloid which they named as "Punarnavine." Later Chopra, Chatterji, and Ghosh (9) found that the alkaloid "Punarnavine" and potassium nitrate were present in both the species, but that the percentages of these substances were greater in the red variety of *Trianthema Portulacastrum* than either in the white variety of this species or in *Boerhaavia diffusa*. Pharmacologically, too, these species have been found to possess more or less similar properties, producing a marked diuresis in cases of edema and ascites.

The therapeutic and chemical alliedness and also the close external resemblance between these two species appear to have been the main cause of confusion in the correct identity of Punarnavā. While many Indian pharmaceutical concerns have employed *Boerhaavia diffusa* for preparations of liquid extracts, etc., of Punarnavā, it is also not uncommon to get samples of *Trianthema Portulacastrum* from drug dealers when Punarnava is required. It was, therefore, decided to investigate the pharmacognostical characteristics of these plants, since no such study has yet been made in a systematic way, in order that these may be distinguished from each other. It may be pointed out, however, that some anatomical observations have already been made on the leaves of *Boerhaavia diffusa* by Sabnis (10), Dastur and Saxton (11), and Bhargava (12), but no detailed account has been given by any of them. So far as the stem anatomy of this species is concerned, the earlier account by Sabnis (10) is very brief. A detailed anatomical account of the stem was, however, published by Maheshwari (13), but the pharmacognostical characteristics were not indicated by him. Furthermore, no study on *Trianthema Portulacastrum* has yet been brought on record. The present investigation, dealing with the pharmacognostical studies of the stem and the leaf, including

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the stomatal index and palisade ratio of both these species, was therefore undertaken.

MATERIALS AND METHODS

Fresh specimens of *Boerhaavia diffusa* and *Trianthema Portulacastrum* were collected from different localities in the Benares Hindu University Campus. Free hand sections were taken from the different portions of leaf and stem and stained in safranin and gentian violet and mounted in Canada balsam in the usual way. The palisade ratio and the stomatal index were determined as described elsewhere (14). To ascertain if the values pertaining to palisade ratio and stomatal index were significantly different from species to species or from locality to locality, the data were statistically analyzed.

EXPERIMENTAL

External Morphology

Boerhaavia diffusa.—It is a diffusely branched, nonsucculent herb, distributed all over India and ascending to 7000 ft. altitude in the warm valleys of the Himalayas. It is found also in Ceylon, Malaya Peninsula, extending to Australia, China, Africa, and America.

The root is stout and fusiform with a woody rootstock. There arise from the crown of the root, numerous stems 2–3 ft. long or even more, slender, prostrate or ascending, swollen at the nodes, and covered with numerous hairs. The leaves are rather thick, opposite, arising in unequal pairs at each node, the larger one being 1–1 1/2 by 3/4–1 inch and the smaller one 1/2–3/4 inch by 1/2 inch. They are broadly ovate, oblong or suborbicular with slightly pointed or rounded apex, and subcordate and rounded base. The margin is entire and subundulate, being turned up and pinkish in certain cases. The upper surface is green and glabrous, but the lower is slightly white. Very often white patches are seen on both surfaces. The petiole is nearly as long as the blade.

The flowers are very small, pedicellate, 4–10 grouped together in small bracteolate umbells, forming slender, long-stalked axillary and terminal panicles. Bracteoles are lanceolate and acute. The perianth tube is about 1/8 inch long, ovoid below, funnel-shaped in the limb, five-lobed, and deep pink in color. The lower part of the tube is strongly ribbed (Fig. 1, *A*). Stamens are 2–3, but sometimes only one and slightly exserted. Ovary is free, strongly stipitate and unilocular with a single basal, erect ovule. It is provided at the top with a filiform style having a peltate stigma. The fruit is 1/8 inch long, clavate, rounded, viscidly glandular on the blunt ribs (Fig. 1, *C*).

Trianthema Portulacastrum.—It is a prostrate, branched, and somewhat succulent herb which grows profusely in the plains with the advent of rains and dies out practically by winter. It is distributed throughout tropical India and Ceylon.

The stem is more or less angular, glabrous or pubescent, and much branched. The leaves are subfleshy, arranged obliquely opposite, in unequal pairs, the upper one being larger about 3/4–1 1/2 inch by 3/4–1 1/4 inch and the lower one 3/8–1/2 inch by 1/4–3/4 inch. They are broadly abovate, rounded, and often apiculate at the apex, cuneate at the base and glabrous. The petiole is 1/4–1/2 inch long, very much dilated and membranous at the base, especially in smaller leaves. These membranous enlargements form a triangular pouch and contain sessile, solitary flowers, fruits, and seeds. Calyx segments are ovate, acute, and calyx tube scarious, thin, closely sheathed by the base of the petiole. Stamens are 10–20. Ovary is obliquely truncate. Style one. Capsule is small, almost concealed in the petiolar pouch, lid truncate, slightly concealed with 2 spreading teeth, carrying away at least 1 seed, and the lower part 2–5 seeded. Seeds are reniform, dull, black, and muricate (Fig. 2, *B*, *C*).

Microscopical Features

Boerhaavia diffusa

Stem.—A transverse section of the young stem shows on the outside a single layer of epidermis, covered externally with cuticle and surrounded by numerous multicellular hairs of the glandular type. There is a deposition of crystalline granules on the epidermal walls below the cuticle. The hairs are uniseriate and consist of a stalk of 8–12 cells and an ellipsoidal or clavate head. The total length of a single hair is 150–220 microns.

The epidermal cells are cubical, measuring 17–20–25 microns by 20–25–32 microns.[1] Below the epidermis is a broad region of cortex consisting of 1–2 layers of collenchyma and 6–7 layers of parenchyma (Figs. 19–22). The cells of collenchyma are present

[1] The measurements of cells and other structures are given ordinarily in three separate figures, of which the first and the third represent the minimum and the maximum values respectively, while the middle indicates the more common value.

Legend to Figures

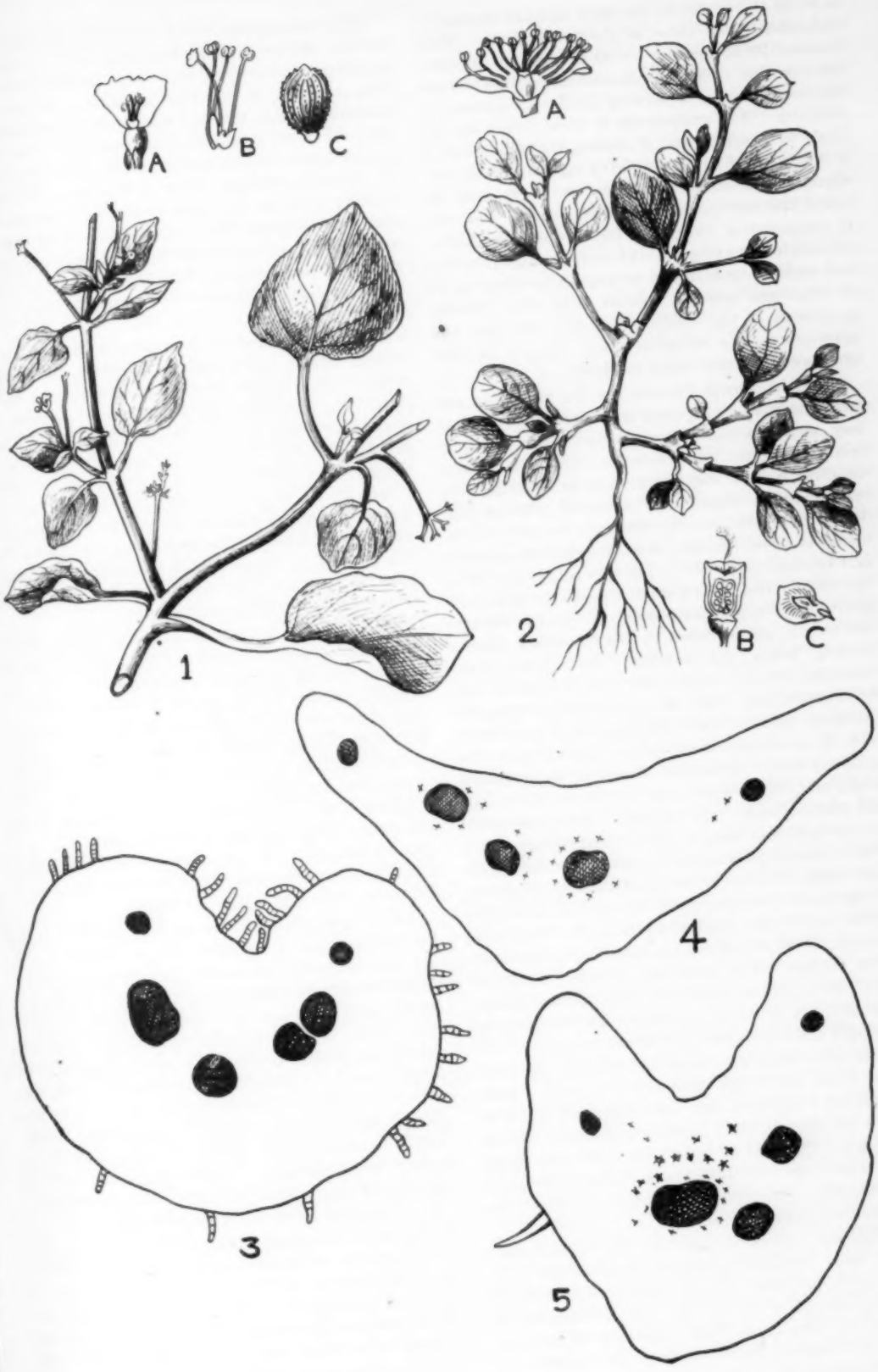
Fig 1.—A part of the plant, *Boerhaavia diffusa*. ×4/5. *A*—Flower dissected. ×13. *B*—Inflorescence. ×1 1/4. *C*—Fruit. ×13.

Fig. 2.—The whole plant of *Trianthema Portulacastrum*. ×1/2. *A*—Flower dissected. ×13. *B*—Ovary cut longitudinally. ×20. *C*—Seed cut longitudinally. ×25.

Fig. 3.—Transverse section of the petiole of *Boerhaavia diffusa*. ×40.

Fig. 4.—Transverse section of the basal part of the petiole of *Trianthema Portulacastrum*. ×40.

Fig. 5.—Transverse section of the upper part of the petiole of the same plant. ×40.


A
B
C
1
A
2
B
C
4
3
5

more at the angles of the stem and are smaller in dimensions than those of the parenchyma. They measure 20–28 microns by 10–20 microns in transverse section. The parenchymatous cells are more or less isodiametric, measuring 20–34–40 microns in diameter. The endodermis is very indistinct, not easily to be made out. Following the endodermis is a thin strip of pericycle of 1–2 layers of cells, surrounding the stele. Often a few fibers may be found scattered beneath the endodermis. The stele is composed of two large bundles in the center, surrounded by a ring of 6–12 loosely arranged bundles and another outer ring of very small bundles, 15–20 or sometimes more in number (Fig. 19). The arrangement of the bundles thus gives the stem the appearance of a monocot stem, except for the presence of intrafascicular cambium.

The two central bundles are the largest in the stem and have a tangential diameter about twice as long as the radial. The secondary xylem elements produced by the intra-fascicular cambium of these bundles, toward the inner side, are arranged in regular radial rows. The secondary phloem produced toward the outside represents the phloem elements of these bundles, since the primary phloem gets crushed very soon. The secondary growth in the middle ring of vascular bundles is also very limited and the primary phloem, like that of the central bundle, gets crushed by the secondary phloem forming below [Maheshwari (13)]. The intrafascicular cambium of the outer ring is belated in its development and joins up to produce a complete cambium cylinder which gives rise later to a broad zone of anomalous wood (Figs. 20 and 21). The cambium cells measure 10–14 microns in tangential length and 5–7 microns in radial width and are 100–125 microns long. The secondary tissue produced by the cambium ring, consists of secondary xylem and secondary phloem in the intra-fascicular region and usually of conjunctive tissue and storage parenchyma in the inter-fascicular region (Fig. 22). The xylem vessels show bordered pits on their walls and measure 17–20–34 microns in diameter in the transverse section. The secondary phloem consists of sieve tubes, companion cells and phloem parenchyma. The sieve tubes measure 110–140 microns in length and 6–1 0 microns in breadth.

The inter-fascicular cambium forms on the inner side, fibers which are similar to substitute fibers, elongated and living and contain starch in them. They measure 10–14–20 microns in diameter and are lignified. The cells formed on the outer side, on the other hand, are thin-walled parenchyma arranged in radial rows. These are much bigger and almost isodiametric in transverse section.

While the secondary tissues are differentiating and their inner products becoming lignified, the outer cells of the pith lying in immediate contact with the lignified tissues of the vascular ring, also become lignified and very hard. The inner cells of the pith, however, remain parenchymatous and measure 50–68–85 microns in diameter.

While the activity of the first cambium begins to decline, additional cambium rings arise by the renewed cambial activity in the outer parenchymatous cells, produced by the first cambium. Outside the primary phloem, this new cambium passes through the cells of the pericycle. The new cambium thus formed functions only for some time and is replaced by another cambium by a similar process, which may be repeated until four or five or more growth rings are produced (Fig. 21). The mode of secondary growth is similar in all the supernumerary cambiums. The secondary xylem and phloem are always found opposite each other so as to give rise to true collateral bundles.

Petiole.—The petiole shows the typical concavo-convex outline in the transverse section and is surrounded on all sides by uniseriate glandular hairs (Fig. 3). The hairs are composed of a stalk of variable number of cells, usually 2–8, and a unicellular glandular head. The head is in most cases ellipsoidal or clavate, and in a few cases spherical (Figs. 10, *A*, *B*, *C*, *D*). The base of the hair is ordinarily formed of a single enlarged epidermal cell. The ellipsoidal head is the largest cell, measuring 55–135 microns in length which is sometimes greater than the length of the stalk. The hair thus has a total length of 120–175–225 microns.

The epidermis is covered by a thick cuticle which is continuous over the hairs as well. The epidermal cells are cubical with greater dimensions on the ventral side (30–56 microns by 22–45 microns) than on the dorsal one (24–40 microns by 20–34 microns). Following the epidermis is a thin layer of collenchyma of one to two layers of cells which are smaller on the dorsal side than on the ventral region. This is followed by a broad zone of parenchyma in which are distributed a number of vascular bundles. The parenchymatous cells are more or less round with distinct intercellular spaces and possess a diameter of 38–60–90 microns in transverse section.

The petiole receives three vascular bundles from the stem forming a dorsal arc in the middle. The two lateral bundles are larger than the central one. These bundles, however, split higher up into five and finally up to seven near the base of the leaf. In such cases, there are two-minute bundles, one on each side of the ventral groove while the remaining ones form a dorsal arc with the central bundle being the largest. Usually on the dorsal side of the bundles, there is some development of the mechanical tissue, which, however, is not distinct in case of the two lateral small bundles. Often the cells of the mechanical layer extend laterally to form a crescent ring outside the dorsal arc of the bundles.

Lamina.—Numerous multicellular hairs of the glandular type, as mentioned above, are present on both the surfaces of the leaf. The hairs with a spherical head and a stalk of four to five cells are more numerous, particularly within the interneural regions, while those with a clavate head and a stalk of 4–8 cells are more predominant on the veins.

The epidermis of the leaf is covered with rather a

thick cuticle which becomes continuous with that of the hair. The epidermal cells on the upper side are more or less cubical, measuring 16–32–40 microns by 24–38–56 microns in the transverse section, while those on the lower side are more or less tabular, being 24–45–56 microns by 24–36–50 microns. The outer walls of the epidermal cells show deposition of crystalline granules of calcium oxalate, beneath the cuticle. At places where this deposit is strongly developed, white patches on the surface of the leaf are formed. This deposition extends also to the walls of the hairs.

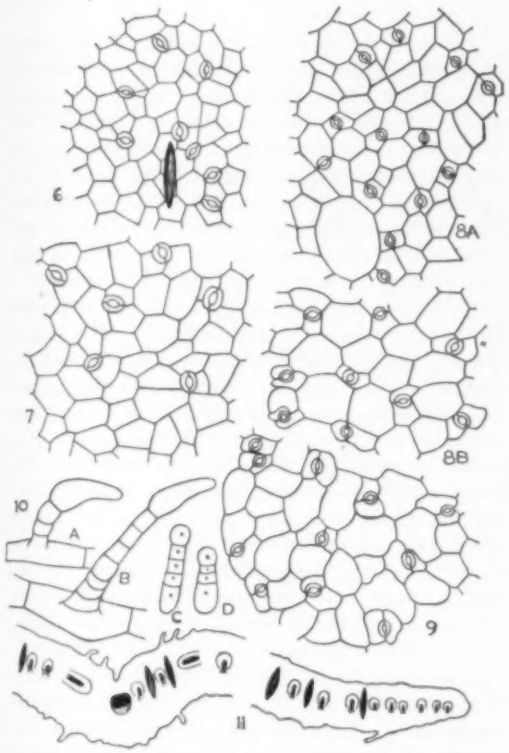


Fig. 6.—Upper epidermis of *Boerhaavia diffusa*, in surface view. ×80.

Fig. 7.—Lower epidermis of the same plant in surface view. ×80.

Fig. 8.—*A*, *B*. Upper epidermis of *Trianthema Portulacastrum* in surface view. ×80. Fig. 8 *A* shows smaller epidermal cells, in which are found bladder-like very big cells.

Fig. 9.—Lower epidermis of *Trianthema Portulacastrum* in surface view. ×80.

Fig. 10.—*A*, *B*, *C*, *D*. Four glandular hairs of *Boerhaavia diffusa*, the first two with a clavate-shaped head and the last two with a spherical head. ×110.

Fig. 11.—Transverse section of the leaf of *Boerhaavia diffusa*. ×20.

Stomata are present on both the surfaces of the leaf (Figs. 6 and 7). They show cuticular ridges but are free from the crystalline granules (Figs. 12 and 13). The distribution of stomata is greater on the upper surface than on the lower one (Table I). They are surrounded by 3–5, but more often by 4 ordinary epidermal cells, of which one is usually smaller than the rest. The stomata are small and measure 24–27–30 microns by $20^1/_2$–22–24 microns with their stomatal aperture $3^1/_2$–5 microns wide.

The epidermal cells in surface view, are all polygonal and straight-walled and measure 28–35–55 microns by 14–30–50 microns. The smaller epidermal cell, surrounding the stomata, however, is 18–22 microns by 9–14 microns.

TABLE I.—NUMBER OF STOMATA, HAIRS, AND EPIDERMAL CELLS IN *Boerhaavia diffusa* PER SQ. MM. LEAF SURFACE

Surface	Stomata	Hairs	Epidermal Cells
Upper	90–154–166	13–21–38	460–704–1014
Lower	66–110–126	11–22–30	302–496–662

The mesophyll is composed of a palisade layer on the upper side and a spongy tissue on the lower side of the leaf. The palisade cells are bent in such a way as to tend to converge round the bundle sheaths (Fig. 12), enclosing the veins. They are shorter in length about 50–56–70 microns, directly above the bundle sheath, but at its sides they become elongated and measure 70–85–90 microns by 12–20–24 microns. Those palisade cells which surround the bundle sheath on the dorsal or lower side of it, are the smallest and measure 32–40 microns by 14–22 microns. The spongy mesophyll consists of 2–4 layers of cells which are loosely arranged and are mostly oval or somewhat irregular, having a length of 30–56–78 microns and a breadth of 28–40–56 microns.

The lamina is traversed by numerous small veins, each surrounded by a bundle sheath which is, however, absent opposite to the phloem of the veins (Fig. 12). There are 4–5 cells of the bundle sheath, visible in the transverse section, mostly with a conical outline, their broad bases being away from the veins and measuring 25–32–40 microns directly above the veins and 45–56–66 microns toward the sides of the veins. However, those surrounding the veins which run longitudinally, appear palisade-like measuring 25–33 microns by 17–22 microns (Fig. 13). Bundles of long raphides traverse the mesophyll with their length lying in a dorso-ventral position (Fig. 11). They occur usually in between the veins and measure 140–170–190 microns in length.

The epidermal cells of the midrib are much smaller as compared to those of the lamina. As in the case of petiole, the epidermal cells are bigger on the ventral side and smaller on the dorsal surface. They measure 16–30–40 microns by 20–24–28 microns on the ventral side and 8–16–22 microns by 12–20–28 microns in the dorsal region. Following the epidermis of the midrib, on the dorsal side, is found a collenchyma of one to 2 layers of cells and a broad zone of parenchyma of 4–6 layers of cells. The cells of the collenchyma have only slightly greater dimensions than those of the epidermal cells,

but the parenchymatous cells are much bigger. The latter are mostly polyhedral or isodiametric with distinct intercellular spaces, measuring 22–38–45 microns in diameter.

A distinct mechanical tissue, 1–3 layers of cells thick, surrounds the vascular bundle of the midrib, dorsally. Near the base of the lamina, the midrib has 3–5 vascular bundles, but higher up it possesses only one bundle. These vascular bundles, however, are not surrounded by a distinct bundle sheath. The phloem of these bundles is composed of sieve tubes, companion cells, and phloem parenchyma, while the xylem consists of annular and spiral and sometimes of reticulate type of vessels and xylem parenchyma. On the ventral side of the vascular bundle, a small amount of parenchyma of 2 to 3 layers of cells or 2–3 palisade-like, but much wider cells, devoid of chloroplasts, are found, below the upper epidermis (Fig. 13).

Trianthema Portulacastrum

The morphological structures observed in both the red-flowered and white-flowered varieties are similar.

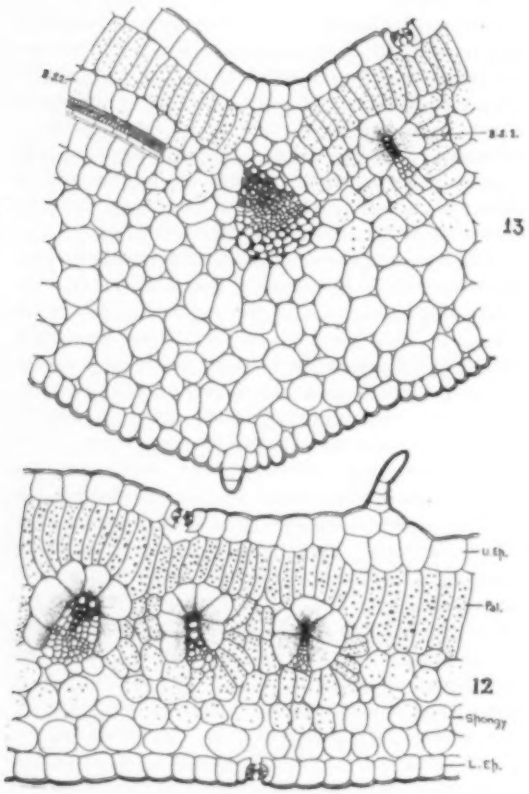


Fig. 12.—Details of the T. S. of lamina of *Boerhaavia diffusa*. ×80.

Fig. 13.—Details of the midrib of the same species. ×80.

U.Ep., upper epidermis; Pal., palisade layer; Spongy, spongy layer; L.Ep., lower epidermis; B.S.1, bundle sheath in transverse view; B.S.2, bundle sheath in longitudinal view.

Stem.—The transverse section of a young internode shows a somewhat plano-convex or slightly biconvex outline (Fig. 17). The single-layered epidermis is covered externally by rather a thin cuticle and numerous unicellular, but very much elongated hairs. These hairs are outgrowths of epidermal cells and measure 60–100–400 microns by 25–30–35 microns. Occasionally bicellular hairs are also found, in which case the second cell has a swollen bulb-like base, situated at the top of the first tapering cell. Rarely a hair may show in the middle region, transverse divisions, giving it a multicellular appearance. All these hairs are nonglandular and the bi- or multicellular ones attain a length up to 700 microns or even more.

Following the epidermis is a broad zone of cortex of 8–10 layers of cells which are parenchymatous with distinct intercellular spaces. In contrast to the epidermal cells which are small and cubical and measure 8–15–18 microns by 14–18–22 microns, the parenchymatous cells are very big and more or less isodiametric with a diameter of 22–50–80 microns. Some of these cells contain rosette crystals of calcium oxalate, 12–20 microns in diameter. A distinct endodermis is, however, not to be made out.

There are present a few layers of pericyclic cells on the outer side of the stele (Fig. 18). The stele is composed of a ring of vascular bundles 20 or more in number. Later when secondary growth takes place, a continuous cambium ring is formed. This cambium produces secondary xylem toward the inner side, and secondary phloem on the outer side opposite to the xylem. In the interfascicular region, however, conjunctive tissue or storage parenchyma is formed. The xylem vessels thus formed are of spiral, reticulate, and pitted type with simple pits on the walls. Sometimes a vessel with bordered pits may also be found. The secondary phloem consists of sieve tube tissue, companion cells, and phloem parenchyma. The conjunctive tissue formed during the secondary growth, in the interfascicular region, becomes thick walled and lignified. The outer cells of the pith lying in the vicinity of the vascular ring also become lignified and thus the stele presents, after secondary growth, a structure resembling that of the outer ring of vascular cylinder of *Boerhaavia diffusa*.

The cells of the pith are very large and parenchymatous with prominent intercellular spaces. They are isodiametric, measuring 70–120–160 microns in diameter. Some of these contain rosette crystals of calcium oxalate.

Petiole.—The outline of the petiole in the basal region of the leaf, is somewhat flat and less concavo-convex, due to the expansion of the sides of the petiole. In the upper region, approaching the lamina, it becomes more concavo-convex (Figs. 4 and 5). Externally it is covered by a very thin cuticle and surrounded by numerous unicellular and nonglandular hairs of the type mentioned above, measuring 60–120–225 microns in length. The epidermal cells on the ventral side are bigger (18–45–56 microns by 14–24–30 microns) than those on the

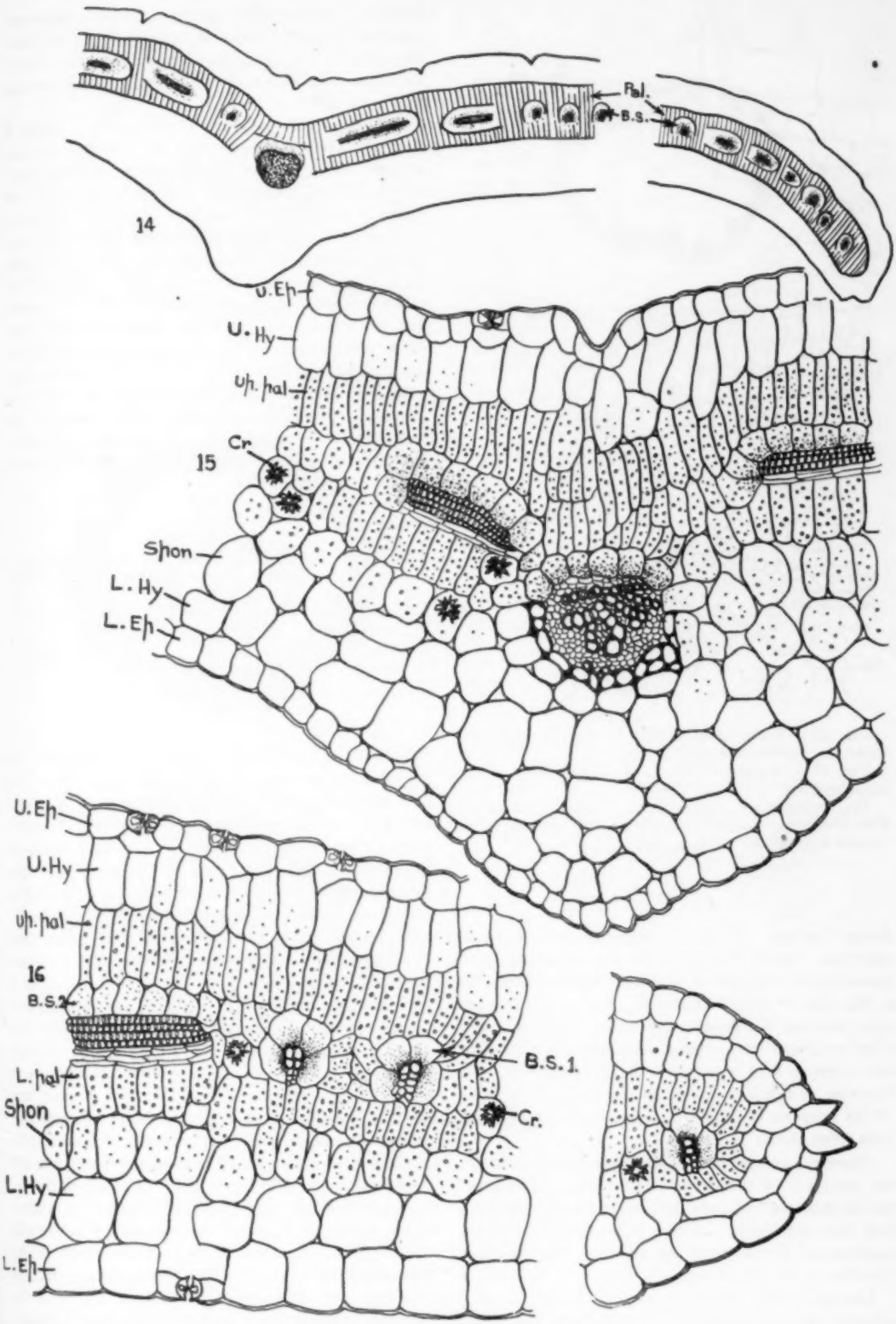


Fig. 14.—Transverse section of *Trianthema Portulacastrum*. ×35.
Fig. 15.—Details of the T. S. of the lamina of the same species. ×150.
Fig. 16.—Details of the T. S. of the midrib of the same species. ×150.
U.Ep., upper epidermis; U.Hy., upper hypodermis; Up.pal., upper palisade layer; L.Hy., lower hypodermis; L.Ep., lower epidermis; B.S.1, bundle sheath in transverse view; B.S.2, bundle sheath in longitudinal view; Cr., rosette crystal of calcium oxalate.

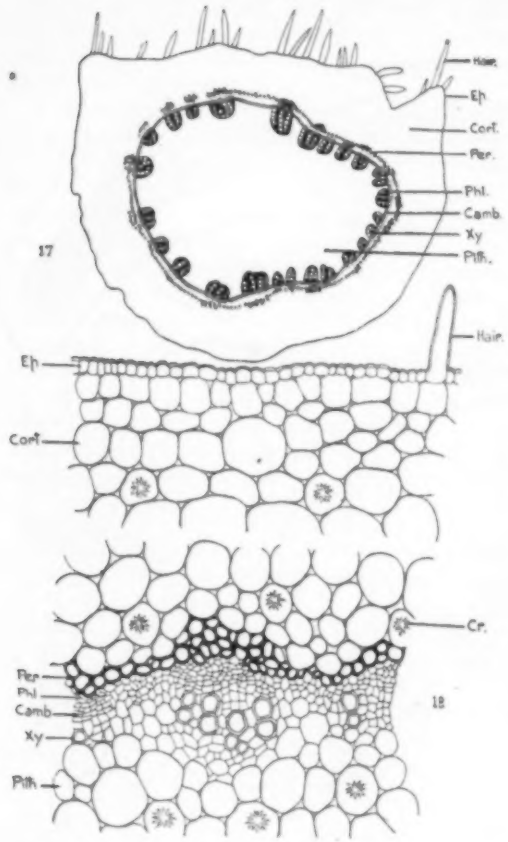


Fig. 17.—Transverse section of the stem of *Trianthema Portulacastrum*. ×20.

Fig. 18.—Details of the T. S. of the stem of the same species. ×150.

Ep., epidermis; Cort., cortex; Per., pericycle; Phl., Phloem; Camb., cambium; Xy., xylem; Cr., rosette crystals of calcium oxalate.

dorsal surface (12–22–28 microns by 15–26–30 microns). Below the epidermis is a broad region of parenchyma, the cells of which are isodiametric with a diameter of 90–120 microns. In the ventral region, however, these parenchymatous cells are somewhat tangentially elongated, with a length of 56–68–100 microns and a breadth of 40–60–80 microns. Crystals of calcium oxalate of the rosette type, 14–18–28 microns in diameter, occur in some of these cells (Figs. 4 and 5).

There are usually five vascular bundles running in the petiole. Of these, the two lateral ones situated on the extreme ends, are the smallest, while the central ones are bigger. There is a poorly developed mechanical tissue often surrounding the vascular bundles on the dorsal side.

Lamina.—The epidermis of the lamina is also covered by a thin cuticle. The cells of the upper epidermis are smaller, measuring 24–56–78 microns by 37–48–64 microns, as compared to those of the lower surface (52–88–112 microns by 52–68–80 microns). A few cells among these are, however, markedly bigger, appearing spherical and bladder-like (Fig. 8, *A*). This is a feature characteristic of Ficoideae, the family to which this plant belongs [Solereder (15)].

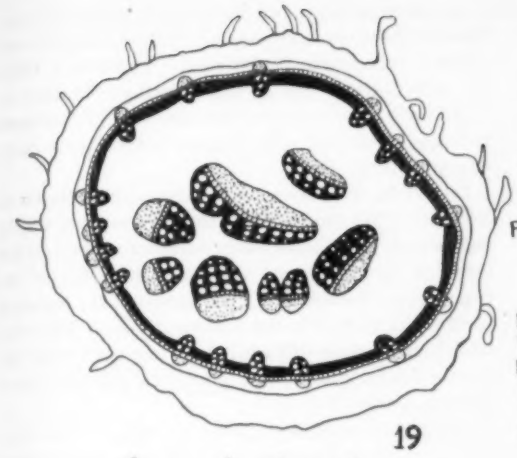
Stomata are found on both the surfaces (Figs. 8 and 9). They are bigger, but fewer in number per sq. mm. leaf area, as compared to those of the *Boerhaavia diffusa* (Table II). They measure 34–38–42 microns by 26–28–32 microns, with the stomatal aperture being 5–10 microns wide. They are surrounded usually by two subsidiary cells, lying with their longer axes parallel to the pore. Of the two subsidiary cells one is often smaller than the other, and when compared with the epidermal cells these are much smaller. While the epidermal cells measure 70–110–150 microns by 38–85–130 microns, the subsidiary cells are 34–50–85 microns by 12–40–68 microns. The lower epidermal cells, however, are bigger than the upper ones. In surface view, these epidermal cells show polygonal, but slightly wavy outline of their cell walls.

TABLE II.—NUMBER OF STOMATA AND EPIDERMAL CELLS IN *Trianthema Portulacastrum* PER SQ. MM. LEAF SURFACE

Surface	Stomata	Epidermal Cells
Upper	56–65–90	268–350–490
Lower	38–40–50	180–2(5–295

Below the epidermis is found a single layer of hypodermis on each side of the leaf (Fig. 16). The cells of the upper hypodermis are more or less palisade-like, 40–58–80 microns by 25–35–50 microns while those of the lower hypodermis are polygonal, measuring 40–58–68 microns by 30–36–50 microns, The mesophyll is composed of a palisade layer on the upper side and a spongy tissue on the lower side. The palisade cells, as in the case of *B. diffusa*, tend to converge around the bundle sheaths, and measure 24–54–75 microns by 10–12–16 microns. Those surrounding the bundle sheath on the dorsal side, are likewise, much smaller, 34–40–53 microns by 10–12–17 microns. The cells of the spongy mesophyll are, however, more compactly arranged than those in case of *Boerhaavia diffusa*, and consist of only one layer. They are in most cases isodiametric with 50–68 microns in diameter. Crystals of calcium oxalate of the rosette type, 17–25 microns in diameter, are occasionally found in the cells of the mesophyll.

Traversing the mesophyll are found numerous veins, each of which is surrounded by a bundle sheath. The structure of the bundle sheath is like that observed in case of *Boerhaavia diffusa*, but in this case only 3–4 cells are found surrounding the veins, in the transverse section. The bundle sheath is absent in the region opposite to the phloem of the veins. The cells of the bundle sheath measure 24–34 microns at their broader (outer) end in the region



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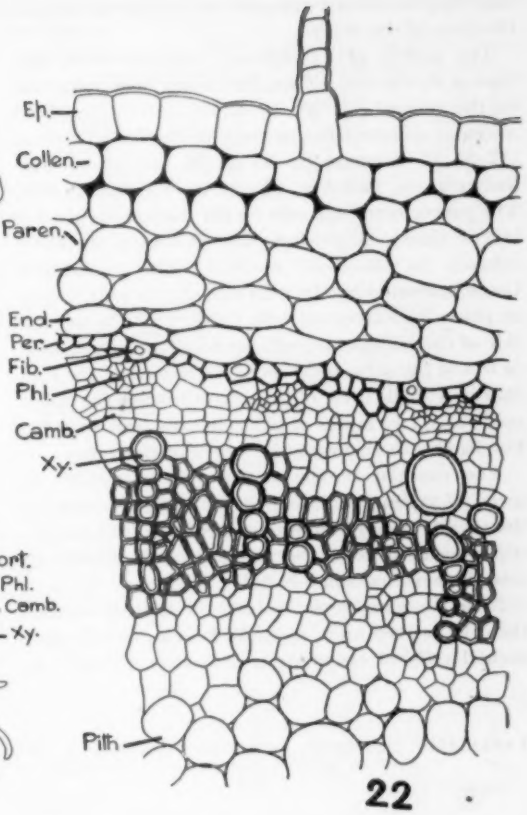

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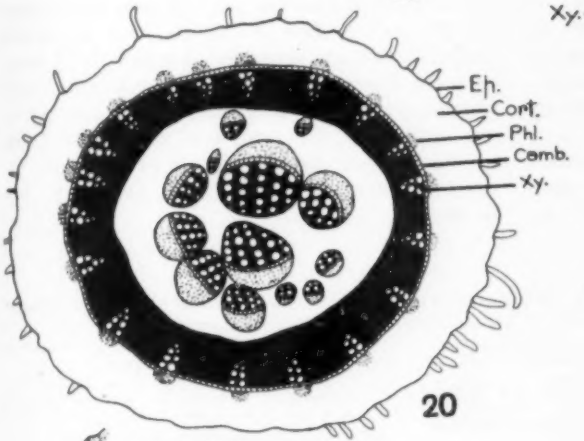

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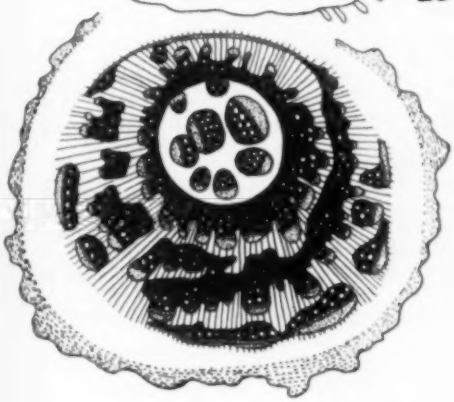

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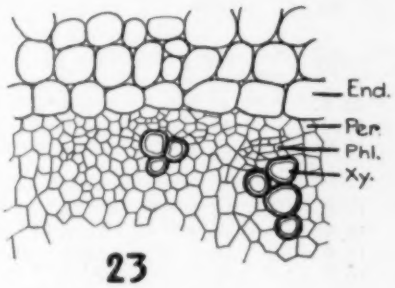

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Fig. 19.—Transverse section of the young stem of *Boerhaavia diffusa*. ×30.

Fig. 20.—Transverse section of the mature stem of the same species. ×30.

Fig. 21.—Transverse section of the mature prostrate stem of the same species, showing greater secondary growth on the lower side than on the upper. ×20.

Fig. 22.—Details of T. S. represented in Fig. 20. ×150.

Fig. 23.—Details of T. S. represented in Fig. 19. ×150.

Ep., epidermis; Collen., collenchyma; Paren., parenchyma; End., endodermis; Per., pericycle; Fib., fiber; Phl., phloem; Camb., cambium; Xy., xylem.

directly above the veins and 48–60 microns toward the sides of the veins.

The midrib of *Trianthema Portulacastrum*, like that of *Boerhaavia diffusa*, has bigger epidermal cells on the ventral side (20–30–42 microns by 28–34–50 microns) as compared to those on the dorsal surface (17–25–35 microns by 14–26–36 microns). Collenchyma is, however, absent on the dorsal side. The parenchymatous cells on the dorsal side are 3–5 layers thick, polyhedral, and measure 30–50–68 microns by 25–34–57 microns. The mechanical tissue, surrounding the vein dorsally, is only one or at places two layers of cells thick. On the ventral side of the midrib, the cells are found arranged more or less in the same order as in the lamina of the leaf, that is, a single layer of hypodermis below the upper epidermis, two layers of palisade cells, and then the bundle sheath enclosing the xylem of the veins.

The vascular bundle of the midrib consists, as usual, of xylem on the ventral side and phloem on the dorsal side. In the lower parts of the leaf, particularly near the base, the vascular bundle breaks up into three or four small bundles.

Near the margin of the leaf, the arrangement of the cells are the same as in the lamina, but the epidermal cells of the margin are often enlarged to form a cone-shaped cell structure (Fig. 16). In the red variety of Trianthema, the epidermal cells of the margin contain a pinkish coloring matter which gives the margin of the leaf a pinkish tinge, characteristic of this variety.

In order to bring out further the diagnostic features of these two species, their stomatal indices and palisade ratios were determined. A glance at Table III, which shows the stomatal indices obtained in both the cases, clearly indicates that *Trianthema Portulacastrum* has much higher values than *Boerhaavia diffusa*.

It will be seen from the analysis of variance in Table III that the variation due to species is highly significant. No significant variance exists either between the upper and the lower surfaces, or between the localities. The interaction between species and surface is also not significant. Thus the two species can be differentiated from each other by their stomatal indices, as also by their palisade ratios given in Table IV.

From the data given above, though it is obvious that *T. Portulacastrum* has significantly higher palisade ratios than *B. diffusa*, yet statistical analysis was made primarily to determine if there were significant differences between localities and whether the interaction between locality and species was significant. The analysis of variance in Table IV clearly shows that the effect of locality or the interaction between locality and species is not significant.

TABLE III.—STOMATAL INDICES OF *Boerhaavia diffusa* AND *Trianthema Portulacastrum* COLLECTED FROM DIFFERENT LOCALITIES

I		II		III		IV[a]	
Upper Surface	Lower Surface	Upper Surface	Lower Surface	Upper Surface	Lower Surface	Upper Surface	Lower Surface
Boerhaavia diffusa							
15.0	14.3	15.1	14.3	15.2	12.7	14.8	12.6
11.7	12.0	14.3	11.3	16.5	17.1	13.6	11.8
12.8	11.2	15.8	18.3	14.3	16.5	15.6	14.6
12.0	13.0	15.2	13.2	12.0	15.8	12.8	15.3
12.9	11.1	16.6	17.5	12.9	13.4	11.9	14.8
Trianthema Portulacastrum							
16.2	17.1	17.5	14.6	20.4	18.6	17.8	16.8
16.4	15.9	19.6	19.3	15.8	19.5	19.5	18.5
14.6	18.7	15.6	15.7	21.1	20.6	18.3	16.0
15.8	16.5	22.4	18.3	19.8	18.8	17.2	17.3
17.6	15.6	21.7	19.4	17.6	19.5	16.5	16.8

Analysis of Variance

Variation Due to	Degrees of Freedom	Sum of Squares	Mean Sum of Squares
Locality	3	75.5210	25.174
Species	1	292.2300	292.230
Surface	1	0.7772	0.7772
Species × surface	1	0.7833	0.7833
Error 1	9	144.5960	16.0662
Error 2	64	62.2025	0.9719
Total	79	576.1100	

[a] Figures I, II, III, and IV indicate four localities.

DISCUSSION

From what has been mentioned above, it will be evident that the two species, namely, *Boerhaava diffusa* and *Trianthema Portulacastrum*, resemble

TABLE IV.—PALISADE RATIOS OF *Boerhaavia diffusa* AND *Trianthema Portulacastrum* COLLECTED FROM DIFFERENT LOCALITIES

	Boerhaavia diffusa — Locality				*Trianthema Portulacastrum* — Locality			
	I	II	III	IV	I	II	III	IV
	6.00	6.50	6.00	6.25	12.00	13.00	12.50	12.00
	4.00	3.50	4.25	3.50	12.50	11.75	13.00	12.75
	4.50	3.25	5.00	4.75	11.25	12.50	13.50	11.50
	3.50	4.50	3.50	4.50	10.25	10.50	12.00	10.00
	4.50	4.00	4.50	5.00	10.50	11.00	13.00	12.00
Mean	4.50	4.35	4.65	4.80	11.30	11.75	12.80	11.65

Analysis of Variance

Variation Due to	Degrees of Freedom	Sum of Squares	Mean Sum of Squares
Locality	3	3.6375	1.2125
Species	1	532.675	532.675
Locality × species	3	3.1875	1.0625
Error	32	31.045	0.9702
Total	39	570.545	

each other in their anatomical features. But for the anatomy of the stem which differs widely in the two cases, inasmuch as there is no anomalous growth in the stem of *T. Portulacastrum* the leaf presents very close similarity in both the species. The occurrence of bundle sheath and the arrangement of palisade layers around it are more or less similar. Even the number of vascular bundles in the petiole of *Boerhaavia diffusa* particularly near the base of the lamina, where the 3 vascular bundles split into 5, is the same as observed in case of *Trianthema Portulacastrum*at similar positions. Despite such close resemblance, there are certain important differences by which the stem and the leaf of these species could be easily distinguished, as shown below:

	Boerhaavia diffusa	*Trianthema Portulacastrum*
	Stem Structure	
1.	There is an outer covering of multicellular, glandular hairs, composed of a stalk of 6–12 cells and a clavate-shaped head.	The hairy covering consists of nonglandular and unicellular hairs of great length. Rarely bi- or multicellular hairs are found.
2.	The epidermal cells are fairly big, so that the ratio of these cells to the parenchymatous cortical cells is less.	The epidermal cells are smaller and the parenchymatous cells are much bigger. Hence the difference between the size of these two types of cells is striking.
3.	There is one to two layers of collenchyma below the epidermis.	Collenchyma is absent.
4.	The stele, in the young stem, consists of two large central bundles, surrounded by a ring of 6–14 loosely arranged bundles and an outer ring of very small bundles 15–20 or more in number. During secondary growth, the outer ring becomes continuous and later, additional cambium rings arise which produce four or five more growth rings.	The stele, in the young stem, consists of a ring of 20 or more small bundles which during secondary growth become continuous.
	Leaf Structure	
5.	The shape of the petiole is of the normal type, being concavo-convex throughout, in the transverse section.	The cross section of the petiole is flattened at the basal region of the leaf, but near the lamina it becomes concavo-convex.
6.	Glandular hairs of the type as found on the stem, but with a shorter stalk, occur on the petiole. The lamina, in the interneural regions, possesses typical glandular hairs 3–4 celled with a spherical head, but on the veins such hairs as have a clavate head are found.	Similar hairs as are found on the stems are present on the petiole as well as the lamina of the leaf.
7.	The petiole is supplied with three vascular bundles which split into five and then into seven, near the base of the lamina.	There are five vascular bundles throughout the petiole.
8.	Stomata, surrounded by 3–5 ordinary epidermal cells, are found on both the surfaces. Both the stomata and epidermal cells are smaller in size, but greater in number per sq. mm. leaf area, the upper surface having 154 stomata and 703 epidermal cells (av.); the lower, 110 stomata and 496 epidermal cells.	Stomata, surrounded by 2 subsidiary cells, are present on both the surfaces. The stomata and epidermal cells are bigger and less in number per sq. mm. leaf area, the upper surface having 65 stomata and 350 epidermal cells at an average, while the lower one has 40 stomata and 205 epidermal cells.

9.	Hypodermis is absent.	A distinct hypodermis is present below each epidermis.
10.	The spongy mesophyll is 2–3 layers thick and loosely arranged.	The spongy mesophyll consists of 1–2 layers of cells which are more compactly arranged.
11.	Bundles of raphides traverse the whole mesophyll dorso-ventrally.	Rosette crystals are found both in the stem and the leaf.
12.	The midrib shows 3–5 vascular bundles in the lower part of the leaf, but higher up there is only one bundle, and the bundle sheath is absent.	There is only one vascular bundle throughout the midrib and it is surrounded by bundle sheath.
13.	The ventral side of the midrib possesses parenchymatous cells.	The ventral side of the midrib has palisade cells like the lamina of the leaf.
14.	The range of stomatal indices and palisade ratios is lower.	This range is higher in *Trianthema Portulacastrum*, as shown in Table V.

TABLE V.—RANGE OF VARIATION IN THE STOMATAL INDICES AND PALISADE RATIOS OF *Boerhaavia diffusa* AND *Trianthema Portulacastrum*

Species	Stomatal Indices		Palisade Ratios
	Upper Surface	Lower Surface	
Boerhaavia diffusa	11.7–13.2–16.0	11.2–14.3–18.3	3.25–4.50–6.25
Trianthema Portulacastrum	14.6–17.2–22.4	15.6–18.3–20.6	10.25–12.00–13.00

SUMMARY

The macro- and microscopical features of the stem and the leaf of *Boerhaavia diffusa* and *Trianthema Portulacastrum* have been studied, with a view to bringing out the distinguishing pharmacognostical features of these species.

The young stem of *Boerhaavia diffusa* is covered externally by characteristic glandular hairs and consists of a layer of epidermis, 1–2 layers of collenchyma, and a broad zone of parenchyma in which vascular bundles are distributed like a monocot stem. There is anomalous wood formed by the renewed activity of the parenchyma formed by the first cambium.

The petiole also is surrounded by similar glandular hairs and consists of 1–2 layers of collenchyma and a broad zone of parenchyma in which are found 3–7 vascular bundles and long raphides of calcium oxalate. The leaf is typically dorsi-ventral with 1 to 2 layers of palisade cells on the ventral side and 3–4 layers of spongy cells on the dorsal side. The vascular bundles are surrounded by bundle-sheaths round which the palisade cells tend to converge. The bundle-sheath is absent opposite to the phloem of the veins. The lower part of the leaf has 3–5 vascular bundles in the midrib, but higher up there is only one bundle. Long bundles of raphides traverse the mesophyll of the leaf.

The young stem of *Trianthema Portulacastrum* is surrounded externally by long, unicellular, and nonglandular hairs which are present also on the petiole and the lamina of the leaf. Collenchyma is absent in the stem, but there are well-developed parenchymatous cells, some of which contain rosette crystals of calcium oxalate. Distributed in the parenchyma is a ring of 20 or more vascular bundles which after secondary growth, become continuous.

The petiole is very much flattened at its base, due to lateral expansions, and contains rosette crystals. There are 5 vascular bundles distributed in the parenchyma of the petiole. The leaf of *Trianthema Portulacastrum* resembles anatomically that of *Boerhaavia diffusa*. It is of dorsi-ventral type with palisade and spongy cells arranged as in *B. diffusa*. The spongy mesophyll is, however, more compact and represented by 1–2 layers of cells which contain also rosette crystals. The veins of the leaf are all surrounded by bundle sheaths which are absent in the phloem portions of the veins.

The two species differ widely in their stomatal indices and palisade ratios, *Trianthema Portulacastrum*, possessing higher values in these regards. Other important differences between the stems and leaves of these two species have been discussed.

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The Estimation of Penicillin K in Commercial Penicillin*

By WILLIAM W. WRIGHT and DONALD C. GROVE

A method for the estimation of penicillin K in commercial penicillin together with its development, calculations, and results obtained on commercial samples is presented.

THE PURPOSE of this paper is to present the experimental data which led to the development of the method now being used by the U. S. Food and Drug Administration for the determination of penicillin K, the calculations involved and some results obtained by the method on commercial penicillin.

METHOD

Dilute a weighed sample or the contents of a vial of penicillin with 0.3 *M* phosphate (Na_2HPO_4 and KH_2PO_4) buffer *p*H 6.0, to give a solution containing approximately 0.6 mg. (1000 units) per ml. In the case of calcium penicillin where a precipitate of calcium phosphate occurs, remove the precipitate by filtration and use the clear filtrate. Place a 15.0-ml. aliquot of this solution in a 125-ml. separatory funnel, add 30.0 ml. of chloroform U. S. P., and shake for one minute. (Carry out all operations at room temperature.) Allow the mixture to stand, with occasional swirling to settle the droplets of chloroform, until the top layer is clear (usually about ten minutes). Draw off all but about 2 ml. of the lower chloroform layer through a small pledget of cotton into a glass-stoppered flask. Take two 4.0-ml. aliquots of the original solution, two 4.0-ml. aliquots of the buffer solution remaining in the separatory funnel, and two 10.0-ml. aliquots of the chloroform solution. Each aliquot is placed in a 125-ml. glass-stoppered Erlenmeyer flask. To one of the two aliquots of each solution add 4.0 ml. of 1 *N* NaOH and allow to stand at room temperature for fifteen minutes. At the end of this time, add 4.0 ml 1.2 *N* HCl and 10.0 ml. of 0.01 *N* I_2 (prepared from 0.1 *N* I_2 U. S. P.). After fifteen minutes titrate the excess iodine using 0.01 *N* $Na_2S_2O_3$ (prepared from 0.1 *N* $Na_2S_2O_3$ U. S. P.). To each of the other aliquots add 10.0 ml. 0.01 *N* I_2 and titrate immediately with 0.01 *N* NaS_2O_3 for the blank determinations. Regard the difference in titers divided by 2.52 as the milligrams of penicillin sodium salt. Calculate the per cent penicillin in the buffer layer and in the chloroform layer as compared to the original solution. The sum of these percentages should be 100% ± 2%. The per cent penicillin K = (96.92 + % in chloroform − % in buffer) × 1.67 (formula A).

The method was developed from observations made by Wattel (1), that at certain hydrogen-ion concentrations penicillin K was extracted by immiscible solvents to a greater extent than the other types of penicillin. Preliminary observations in our laboratories showed that when a 0.3 *M* phosphate buffer solution of crystalline penicillin K[1] at *p*H 5.0 was shaken with two volumes of chloroform, the penicillin K distributed itself so that about 75% was found in the chloroform while about 25% remained in the aqueous phase. Crystalline penicillin G (FDA working standard) under the same conditions

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[1] Manufactured by Charles Pfizer and Company, Inc., and obtained through the courtesy of the Antibiotics Study Section, U. S. Public Health Service.

distributed itself so that about 14% was found in the chloroform and about 86% remained in the aqueous phase. Experiments at higher hydrogen-ion concentrations were carried out to determine whether a point could be reached where a fairly high percentage of penicillin K could be extracted with little or no penicillin G: at *p*H 5.5 the chloroform extracted about 60% penicillin K and 5% penicillin G, at *p*H 6.0 about 31% K and 1.5% G, and at *p*H 6.5 about 11% K and 0.7% G. Extraction at *p*H 6.0 was chosen because at *p*H 5.5 too much G was extracted and at *p*H 6.5 too little K was extracted. The iodometric assay method of Alicino (2) as modified by Mundell, *et al.* (3) for determining the potency of the penicillin was chosen because the chloroform extract could be assayed directly without an additional shakeout into buffer as is required when the bioassay method is used.

Table I shows the results obtained by the above method using penicillin K alone and penicillin G alone.

TABLE I.—DISTRIBUTION RESULTS OBTAINED AT *p*H 6.0 ON PENICILLIN K AND G

Penicillin	% in Buffer Layer	% in $CHCl_3$	% Recovery
K	68.36	28.22	96.58
K	68.76	32.10	100.86
K	67.92	32.77	100.69
K	67.99	31.93	99.92
K	69.77	32.48	102.25
Av. of 5	68.56	31.50	100.06
G	98.06	1.14	99.20
G	97.40	1.53	98.93
G	97.80	1.45	99.25
G	96.33	1.95	98.28
Av. of 4	97.40	1.52	98.92

It will be noted that the average recovery in both layers in the case of penicillin K was 100.06% and in the case of penicillin G 98.2%. Correcting the figures on the basis of 100% recovery, the following distribution figures are obtained.

Penicillin	% of Total in Aqueous Layer	% of Total in $CHCl_3$
K	68.52	31.48
G	98.46	1.54

Having established the distribution figures for penicillin K and G, the percentage of penicillin K in an unknown sample of penicillin can be calculated on a weight basis from either of the following formulas.

$$\% \text{ K} = \frac{(98.46 - \% \text{ in aqueous})}{(98.46 - 68.52)} \times 100 \text{ or}$$

$$(98.46 - \% \text{ in aqueous}) \; 3.340 \quad \text{(B)}$$

$$\% \text{ K} = \frac{(\% \text{ in chloroform} - 1.54)}{(31.48 - 1.54)} \times 100 \text{ or}$$

$$(\% \text{ in chloroform} - 1.54) \; 3.340 \quad \text{(C)}$$

In actual practice the sum of the amount in the chloroform layer and the amount in the buffer layer is not always exactly 100%, therefore there will be some variation in the per cent of penicillin K depending upon whether one calculates this percentage from the amount remaining in the aqueous buffer solution or the amount extracted by the chloroform using the above two formulas. For this reason an average of the sum of the per cent K obtained by both of the above formulas is used to obtain the per cent K, as expressed by the following:

$$\% \text{ K} = (96.92 + \% \text{ in chloroform} - \% \text{ in buffer}) \times 1.67 \quad \text{(A)}$$

Separate solutions of penicillin G and penicillin K were prepared in 0.3 *M* phosphate buffer *p*H 6.0 to give a concentration of approximately 0.6 mg. per ml. Each solution was assayed for milligrams per milliliter by the iodometric method and then the two solutions were mixed in varying proportions and the mixtures assayed for the per cent of penicillin K by the above method. The results obtained are shown on Table II.

TABLE II.—RESULTS OBTAINED ON MIXTURES OF PENICILLIN K AND G

Known K and G in Mixture, %		Penicillin Found in Each Layer, %		Penicillin K Found Using Formula A, %
K	G	In Buffer	In $CHCl_3$	
0	100	98.46	1.54	0.0
10.2	89.8	95.49	4.51	9.9
21.4	78.6	93.68	7.20	17.4
36.2	63.8	86.76	11.72	36.5
49.8	50.2	83.39	16.61	50.3
100	0	68.52	31.48	100.0

It was desirable next to determine what effect the other penicillin —X, F, and dihydro F—would have on the method for the determination of penicillin K. Accordingly, these different types of penicillin were each run through the procedure separately and the per cent in the buffer and in the chloroform determined. Table III gives a summary of the amount found in each layer for each type penicillin. It will be noted that penicillin X will not interfere. Penicillins F and dihydro F are somewhat more soluble in chloroform than penicillin G; however, the error introduced by the presence of these two types of penicillin is very small.

TABLE III.—DISTRIBUTION OF VARIOUS PENICILLINS BETWEEN CHLOROFORM AND 0.3 *M* *p*H 6.0 BUFFER

Penicillin	Penicillin Found in Each Layer, %		Using Formula A, % K Apparently Present
	In Buffer	In $CHCl_3$	
G	98.46	1.54	0.0
X[a]	98.52	1.00	1.0
F[a]	96.28	2.16	4.7
Dihydro F[a]	96.18	2.33	5.2
K	68.52	31.48	100.0

[a] The penicillin F was manufactured by the Upjohn Company and the penicillin dihydro F by Chas. Pfizer and Company, Inc. Both samples were obtained through the courtesy of the Antibiotics Study Section, U. S. Public Health Service. The penicillin X was obtained through the courtesy of Lederle Laboratories.

Tables IV and V give some typical penicillin K results obtained on commercial lots of sodium and calcium penicillin, respectively. It may be said that the penicillin K content of penicillin being distributed at the present time is very small.

TABLE IV.—SODIUM PENICILLIN

Manufacturer	Lot No.	Date Tested	% K
A	703A007	5-1-47	0.0
	704A001	5-12-47	2.5
	704A002	5-26-47	1.9
	705A003	6-9-47	3.2
	705A004	6-11-47	3.7
B	5479	5-22-47	2.8
	54715	5-22-47	0.0
	54719	5-30-47	0.2
	54726	6-5-47	1.4
	6475	6-13-47	0.0
C	C7690	5-8-47	0.0
	E7688	5-23-47	1.6
	E7687	5-23-47	2.2
	E7690	5-26-47	3.2
	E7616	6-11-47	5.1
D	1502	7-4-47	0.0
	1513	7-4-47	6.5
	1514	7-4-47	7.9
	1517	7-4-47	6.7
	1518	7-4-47	5.3
E	3810-837A	6-25-47	5.3
	3810-891A	6-30-47	7.5
	7810-881C	7-1-47	10.4
	7810-881D	7-2-47	12.1
	7810-877D	7-3-47	5.1
		Av.	3.8

TABLE V.—CALCIUM PENICILLIN

Manufacturer	Lot No.	Date Tested	% K
A	705X902	6-11-47	2.1
	706X900	6-17-47	0.0
	706A001	6-23-47	1.2
	706X901	6-25-47	2.2
	706X902	7-4-47	0.0
B	1591	5-26-47	5.7
	1701	6-11-47	5.5
	1702	6-11-47	1.8
	1703	6-14-47	1.4
	1704	6-14-47	4.3
C	1117C	5-3-47	0.0
	1427C	6-19-47	2.1
	1457C	6-23-47	2.2
	1477C	6-25-47	0.8
	1517C	7-1-47	5.2
D	1505	6-5-47	1.7
	1527	6-24-47	2.3
	1528	6-24-47	4.4
	1533	6-25-47	5.6
	1534	6-25-47	0.3
E	2S245	6-10-47	1.7
	18S177	6-10-47	4.8
	18S178	6-11-47	0.0
	2S247	6-19-47	7.1
	2S248	7-5-47	5.0
F	471952	6-24-47	11.4
	472916	6-25-47	6.8
	472917	7-2-47	14.1
	472918	7-4-47	5.5
	471957	7-4-47	18.8
G	CO619	6-17-47	0.0
	CO677	6-19-47	0.0
	CO686	6-25-47	0.0
	CO749	6-27-47	2.3
	CO759	7-3-47	0.7
		Av.	3.6

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(2) Alicino, J. F., *Ind. Eng. Chem., Anal. Ed.*, **18**, 619 (1946).

(3) Mundell, M., Fischback, H., and Eble, T. E., THIS JOURNAL, **35**, 373(1946).

WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

Montanic acid
Glucoascorbic acid
Acenaphthylene
Cuscohygrine
Hemipinic acid
Hordenine
4-Hydroxyquinoline-3-carboxylic acid
4-Phenyl-2-aminobutyric acid
Undecyl aldehyde
Scopoletin
N-Methylnipecotic acid
Ethyl vanillate
Thiocytosine
Phosphatase, purified
Hydroxylamine-O-acetic acid hydrochloride
Methoxinine
d-Erythrose
d-Threose
17-Hydroxycorticosterone
11-Dehydro-17-hydroxycorticosterone
Stigmasterol
Maltobionic acid

The Preparation of Certain Substituted Alkenols and Their Chloro Analogs*

By FRED SEMENIUK† and GLENN L. JENKINS

CERTAIN olefinic halogenated compounds are of significance from the standpoint of preparative methods proposed for them and of their possible utility as intermediates in the synthesis of new types of physiologically active compounds. These halogenated compounds are the 4-substituted-1-halo-2-butenes, which are satisfied by the general formula: $Q—CH_2—CH=CH—CH_2—X$, where Q— is any substituent not necessarily hydrocarbonaceous. Those selected for preliminary study constitute a fairly uniform series of five in which the various values of Q— are methyl-, cyclohexyl-, phenyl-, α-naphthyl- and *o*-ethoxyphenyl-, and in which the halo-substituent is chloro-.

3,4-Epoxy-1-butene (Butadiene monoxide), $H_2C\overset{O}{\diagup\diagdown}CH—CH=CH_2$, is a compound of fair availability in recent years. Its reactive and especially additive properties are many and variable. Among many others, it will add Grignard reagents—a characteristic of epoxy compounds; the manner in which this addition takes place with respect to the direction of opening of the epoxy ring is debatable, so that on hydrolysis subsequent to addition, the formation of either or both of two possible alcohols must be considered, corresponding to the structures:

$$(a)\quad HO—CH_2—\underset{\displaystyle Q}{\underset{|}{C}}H—CH=CH_2$$

2-Substituted-3-butenol

and

$$(b)\quad Q—CH_2—\underset{\displaystyle OH}{\underset{|}{C}}H—CH=CH_2$$

1-Substituted-3-buten-2-ol
(Vinyl-substituted-methyl-carbinol)

where Q—Mg—X is the Grignard reagent employed.

Using methylmagnesium iodide as the Grignard reagent, adding to 3,4-epoxy-1-butene, and following the addition with hydrolysis (acid catalyzed), we obtained a pentenol whose constants (b. p. 133–139°/747 mm.; m. p. of 3,5-dinitrobenzoate: 59–60°) corresponded to neither 2-methyl-3-butenol (isomeric form *a*), (b. p. 120–121°/756 mm.; m. p. of 3,5-dinitrobenzoate: 57.5–58°) (1), nor 1-methyl-3-buten-2-ol (vinylethylcarbinol) (isomeric form *b*), (b. p. 111–118°/ordinary, or no pressure specified) (2–6). Our pentenol, however, did correspond to the constants (b. p. 138–142°/no pressure specified) (5, 7–9) for a structure of the isomeric form: $H_3C—CH_2—CH=CH—CH_2—OH$ (4-methyl-2-butenol, 2-pentenol, β-ethylallyl alcohol).

A reasonable explanation for the formation of 4-methyl-2-butenol, but not of 2-methyl-3-butenol (which could not be isolated), is that a combination of two events is involved: (*a*) the epoxy ring of 3,4-epoxy-1-butene opens to Grignard reagents to form, preferentially, secondary alcohols when the addition product is hydrolyzed; (*b*) if the hydrolysis is acid catalyzed, the secondary alcohols undergo allylic rearrangement to β-substituted-allyl alcohols which correspond to the 4-substituted-2-butenols; these in turn are obvious precursors for the preparation of the 4-substituted-1-halo-2-butenes.

Allylic rearrangements have been experimentally established for a variety of susceptible classes of compounds, among which are the vinylalkylcarbinols, vinylarylcarbinols, and vinyldialkylcarbinols. The general equation by which the rearrangement may be represented is:

$$—\underset{\displaystyle OH}{\underset{|}{C}}—CH=CH_2 \rightarrow —C=CH—CH_2—OH$$

Delaby (10) and Prevost (5, 8, 11) have described its application to vinylalkylcarbinols. An extension of the rearrangement

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is applicable in terms of the more general equation:

representing the preparation of rearranged analogs. The effective agents of conversion and of rearrangement of this type are HX, PCl_3, PBr_3, and $SOCl_2$ (12–14).

The 1,4-addition of Grignard reagents to 3,4-epoxy-1-butene cannot be denied as a possible reaction mechanism to explain the formation of 4-substituted-2-butenols without relying entirely upon the incidence of allylic rearrangement. Furthermore, it serves to explain the fact that alcohols of but one isomeric form could be isolated.

3,4-Epoxy-1-butene proved to be an interesting intermediate in the route of synthesis employed. Certain difficulties were encountered in the manipulative procedure, the most notable of which were the extreme exothermy, high viscosity and foaming encountered in the reaction mixtures during the addition of the 3,4-epoxy-1-butene to the prepared Grignard reagents. It was found more desirable to employ less cooling of the reaction flask at the expense of time required for adding the 3,4-epoxy-1-butene to the Grignard reagent, in order to obviate the viscosities which made stirring both difficult and dangerous from the standpoint of sudden foaming which resulted and of the incidence of violent local action due to inadequate stirring.

The five alcohols prepared have been assigned the same general formula, corresponding to the isomeric form represented by the name 4-substituted-2-butenol, for the reason that the same general procedure was used in the preparation of all of them. However, minor variations in the procedure were necessary for certain individual members.

Of the five alcohols, the analogs of radical weight greater than methyl- are new. They were derivatized as their 3,5-dinitrobenzoates; the derivatives were analyzed for % N, the results checking satisfactorily with calculated values.

For the conversion of the five 4-substituted-2-butenols to their corresponding chloro analogs, the 4-substituted-1-chloro-2-butenes, thionyl chloride was used as the conversion agent. No rearrangements were anticipated since thionyl chloride favors the same isomeric form (14).

Of the five 4-substituted-1-chloro-2-butenes prepared, the analogs of radical weight greater than methyl- are new. The series showed a notable tendency to decompose with the liberation of HCl. This was demonstrated by their precipitation of AgCl immediately in the cold with aqueous or alcoholic silver nitrate solution, by formation of dimethylammonium chloride exclusively, on reaction with dimethylamine, and by consistently low (1–4 percentage points) analyses for % Cl. Attempts to prepare Grignard reagents of them for derivation of their anilides intended for analyses for % N to complement the % Cl analyses, failed, even under the strenuous conditions recommended by Underwood and Gale (15).

Inasmuch as R—Br compounds generally form Grignard reagents more readily than do the corresponding R—Cl compounds (16, 17), and as 3,4-epoxy-1-butene has proved to be a satisfactory intermediate in the preparation of this class of compounds, it is proposed to prepare the bromo-analogs of the 4-substituted-1-chloro-2-butenes.

EXPERIMENTAL

In the preparation of the 4-substituted-2-butenols ground glass equipment and a motor-driven mercury-seal stirrer were used.

1. **4-Methyl-2-butenol (2-Pentenol, β-Ethylallyl alcohol) (I).**—To 300 cc. anhydrous ether containing 48.6 Gm. (2 atoms) magnesium turnings and a crystal of iodine was added 284 Gm. (2 moles) methyl iodide at a rate governing smooth Grignard reagent formation. Mild heat and stirring were applied for 8 hours after spontaneous reaction. While keeping the reaction flask efficiently cooled in an ice-salt bath, 140 Gm. (2 moles) 3,4-epoxy-1-butene was added dropwise with stirring. Violent reaction, liberation of heat and dense white fumes, and extreme foaming occurred. These were governed by interruptions in additions and stirring. The total addition took eight hours. The bath was removed and stirring was continued for six hours. The reaction mixture was poured onto 1 Kg. of cracked ice and hydrolyzed by stirring into it 1 L. diluted H_2SO_4. The ether layer was salted out, separated, and evaporated free of ether. The resi-

due was dried with anhydrous $CaCl_2$, separated, and distilled. The fraction boiling steadily distilled at 133–139°/747 mm., 50–55°/12 mm. A pale yellow liquid with a pungent, garlic odor.

Yield: 61.4 Gm. (35.7%); $d_4^{20} = 0.859$; $n_D^{25} = 1.4325$. M. p. of 3,5 dinitrobenzoate 59–60°.

Anal.—Calcd. for 3,5-dinitrobenzoate ($C_{12}H_{12}O_6N_2$): N, 10.00. Found: N, 10.45, 10.54.

2. 4-Cyclohexyl-2-butenol (II).—Using the same general procedure as for the preparation of (I), 165.5 Gm. (1.4 moles) chlorocyclohexane, 34.0 Gm. (1.4 atoms) magnesium turnings and 98 Gm. (1.4 moles) 3,4-epoxy-1-butene yielded, when the dried residue from the evaporation of the ether was submitted to purification by vacuum distillation (glass wool was used in the distillation flask to prevent violent bumping and obtain steady distillation) the fraction boiling steadily at 115–120°/13 mm. A pale yellow liquid.

Yield: 73.3 Gm. (34%); $d_4^{20} = 0.939$; $n_D^{25} = 1.4795$. M. p. of 3,5 dinitrobenzoate 106–107°.

Anal.—Calcd. for 3,5-dinitrobenzoate ($C_{17}H_{20}O_6N_2$): N, 8.05. Found: N, 8.09, 8.16.

3. 4-Phenyl-2-butenol (III).—Using the same general procedure as for the preparation of (I), 314 Gm. (2 moles) bromobenzene, 48.6 Gm. (2 atoms) magnesium turnings and 140 Gm. (2 moles) 3,4-epoxy-1-butene yielded, when the dried residue from the evaporation of the ether was submitted to purification by vacuum distillation, the fraction boiling steadily at 120–125°/12 mm. The product is a pale yellow liquid.

Yield: 112.7 Gm. (38%); $d_4^{20} = 1.006$; $n_D^{25} = 1.5390$. M. p. of 3,5 dinitrobenzoate 104–105°.

Anal.—Calcd. for 3,5-dinitrobenzoate ($C_{17}H_{14}O_6N_2$): N, 8.19. Found: N, 8.66, 8.47.

4. 4-α-Naphthyl-2-butenol (IV).—To 300 cc. anhydrous ether containing 41 Gm. (1.68 atoms) magnesium turnings and a crystal of iodine was added 349 Gm. (1.68 moles) α-bromonaphthalene at such a rate as to keep Grignard reagent formation progressing. Following spontaneous reaction, which was incomplete, as strong heat as was safe, and moderate stirring were applied for twenty-four hours. The reaction flask was immersed in an ice bath and 117.6 Gm. (1.68 moles) 3,4-epoxy-1-butene was added dropwise with stirring. The reaction was violent (dense white fumes and heat, but no foaming) and was governed by interruptions in addition and stirring. The total addition took twelve hours. The ice bath was removed and stirring was continued for twelve hours. The reaction mixture was poured onto 1 Kg. of cracked ice and hydrolyzed with 1 L. diluted H_2SO_4. The ether layer was salted out, separated, and evaporated free of ether. The residue was dried with anhydrous $CaCl_2$, separated, and distilled. The fraction boiling steadily distilled at 163–168°/3 mm. A viscous, pale yellow liquid.

Yield: 100.5 Gm. (30.2%); $d_4^{20} = 1.114$; $n_D^{25} = 1.6168$. M. p. of 3,5 dinitrobenzoate 179–180°.

Anal.—Calcd. for 3,5-dinitrobenzoate ($C_{21}H_{16}O_6N_2$): N, 7.15. Found: N, 7.57, 7.73.

5. 4-*o*-Ethoxyphenyl-2-butenol (V).—To 300 cc. anhydrous ether containing 48.6 Gm. (2 atoms) magnesium turnings and a crystal of iodine was added 313.2 Gm. (2 moles) *o*-chlorophenetole at such a rate as to keep Grignard reagent formation progressing. Great difficulty was encountered in attempting to get the Grignard reagent to form; the addition of methyl iodide and of more iodine, and finally the application of strong heat and constant stirring for forty-eight hours had to be resorted to. At the end of this time almost all the magnesium had reacted. The reaction flask was cooled and 140 Gm. (2 moles) 3,4-epoxy-1-butene was added slowly, dropwise with stirring. The amount of cooling was only slight, since excessive cooling made the reaction mixture too viscous for stirring. Total addition took eight hours. Stirring was continued six hours longer with the application of mild heat to maintain the fluidity of the reaction mixture. The reaction mixture was hydrolyzed in the usual way, using 1 Kg. cracked ice and 1 L. diluted H_2SO_4. This was followed by salting out, separation, evaporation of the ether, drying of the residue, separation, and finally purification by vacuum distillation. The fraction boiling steadily distilled at 132–137°/3 mm. The product is a viscous, pale yellow liquid.

Yield: 56.5 Gm. (14.7%); $d_4^{20} = 1.040$; $n_D^{25} = 1.5377$. M. p. of 3,5 dinitrobenzoate 79°.

Anal.—Calcd. for 3,5-dinitrobenzoate ($C_{19}H_{18}O_7N_2$): N, 7.26. Found: N, 7.99, 7.72.

6. 4-Methyl-1-chloro-2-butene (1-Chloro-2-pentene, β-Ethylallyl chloride) (VI) (18, 19).—Ninety-three grams (0.783 mole) thionyl chloride was placed in a 200-cc. flask equipped with a dropping funnel and a reflux condenser bearing a calcium chloride tube and brought to a gentle boil on a steam bath. Fifty-seven and two-tenths grams (0.665 mole) 4-methyl-2-butenol (I) was added dropwise over a period of one hour. Gentle heating was continued for four hours after total addition. The reaction mixture was washed several times with successive portions of water and finally with a 5% $NaHCO_3$ solution. The dark brown liquid was dried with anhydrous $CaCl_2$, separated and distilled. The fraction boiling steadily distilled at 98–103°/747 mm. A colorless liquid, darkening rapidly with an increasing pungent odor of evolved HCl.

Yield: 35.2 Gm. (50.7%); $d_4^{20} = 0.892$; $n_D^{25} = 1.4290$.

Anal.—Calcd. for C_5H_9Cl: Cl, 33.93. Found: Cl, 32.42, 31.67.

7. 4-Cyclohexyl-1-chloro-2-butene (VII).—Using the same general procedure as for the preparation of (VI), 62 Gm. (0.52 mole) thionyl chloride and 68.3 Gm. (0.443 mole) 4-cyclohexyl-2-butenol (II) yielded the fraction boiling steadily at 103–108°/14 mm. A colorless liquid darkening rapidly with an increasing pungent odor of evolved HCl.

Yield: 32.9 Gm. (43%); $d_4^{20} = 0.964$; $n_D^{25} = 1.4765$.

Anal.—Calcd. for $C_{10}H_{17}Cl$: Cl, 20.54. Found: Cl, 16.97, 16.49.

8. 4-Phenyl-1-chloro-2-butene (VIII).—Using the same general procedure as for the preparation of (VI), 100 Gm. (0.845 mole) thionyl chloride and 106.4 Gm. (0.72 mole) 4-phenyl-2-butenol (III) yielded the fraction boiling steadily at 107–112°/12 mm. A colorless liquid darkening slowly with an increasing pungent odor of evolved HCl.

Yield: 58.5 Gm. (49%); d_4^{20} = 1.032; n_D^{25} = 1.5365.

Anal.—Calcd. for $C_{10}H_{11}Cl$: Cl, 21.28. Found: Cl, 20.31, 19.23.

9. 4-α-Naphthyl-1-chloro-2-butene (IX).—Using the same general procedure as for the preparation of (VI), 76.3 Gm. (0.64 mole) thionyl chloride and 107.8 Gm. (0.545 mole) 4-α-naphthyl-2-butenol (IV) yielded the fraction boiling steadily at 162.5–167.5°/7 mm. A pale yellow liquid becoming more yellow on standing with an increasing pungent odor of evolved HCl.

Yield: 39.7 Gm. (33.8%); d_4^{20} = 1.139; n_D^{25} = 1.6158.

Anal.—Calcd. for $C_{14}H_{13}Cl$: Cl, 16.37. Found: Cl, 14.54, 14.45.

10. 4-o-Ethoxyphenyl-1-chloro-2-butene (X): Using the same general procedure as for the preparation of (VI), 38.6 Gm. (0.324 mole) thionyl chloride and 52.9 Gm. (0.276 mole) 4-*o*-ethoxyphenyl-2-butenol (V) yielded the fraction boiling steadily at 120–125°/4 mm. A pale yellow liquid darkening on standing with an increasing pungent odor of evolved HCl.

Yield: 26 Gm. (44.6%); d_4^{20} = 1.077; n_D^{25} = 1.5246.

Anal.—Calcd. for $C_{12}H_{15}OCl$: Cl, 16.84. Found: Cl, 16.59, 16.14.

SUMMARY

1. An evaluation has been made of the utility of 3,4-epoxy-1-butene (butadiene monoxide) as an intermediate in the preparation of 4-substituted-2-butenols and of their chloro analogs.

2. A series of five analogous 4-substituted-2-butenols in which the substituents are methyl-, cyclohexyl-, phenyl-, α-naphthyl-, and *o*-ethoxyphenyl- has been prepared, described, and derivatized as their 3,5-dinitrobenzoates. The derivatives have been analyzed for % N, with satisfactory results. All analogs, except that in which the substituent is methyl-,(2-pentenol, β-ethylallyl alcohol), are new.

3. A series of five analogous 4-substituted-1-chloro-2-butenes in which the substituents are methyl-, cyclohexyl-, phenyl-, α-naphthyl-, and *o*-ethoxyphenyl- has been prepared, described, and analyzed for % Cl, with indicative results. All analogs, except that in which the substituent is methyl-, (1-chloro-2-pentene, β-ethylallyl chloride), are new.

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WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

Methionine sulfone
Ketomethionine
4-Diethylamino-2-nitrosophenol
Thyroglobulin
3,3′-Dimethoxybenzidine-5,5′-disulfonic acid
N-(4-azobenzenenaphthyl-1) ethylene diamine
Methoxinine
Guaiacol-4,6-dialdehyde
Guaiacol-4,6-dicarboxylic acid
4-Menthene-3-one
Indole-3-aldehyde
Tryptamine
2-Hexenal
Glucamine
d-Erythrose
d-Threose
Enol phosphopyruvic acid
Piperonylacetonitrile
4-Hexene-2-one
3-Geranylcatechol

Acute Toxicity of Arsenate of Lead in Animals*†

By J. L. VOIGT, L. D. EDWARDS, and C. H. JOHNSON

The oral LD_{50} of lead arsenate in a single dose was determined for several species of animals. For chickens it was calculated that the LD_{50} was 450 mg./Kg. In white rats the LD_{50} of lead arsenate was found to be 825 mg./Kg. For rabbits the LD_{50} was calculated as 125 mg./Kg.

ARSENATE OF lead is a poison used commonly in agriculture as a spray. Because of the possibility of farm animals ingesting lead arsenate residues when feeding on or near vegetation that has been sprayed, much interest has been shown in the toxicity of this compound. Since the quantity of residue ingested at any one time usually is small, much of the work done has dealt only with chronic poisoning. Very few investigators have attempted to determine the size of the single oral dose of lead arsenate which would cause death in higher animals. This paper presents some work done with the latter purpose in view.

Thomas and Shealy (1), in reporting their results with chickens, stated that there appeared to be no definite correlation between the weights of the birds and a lethal dose of lead arsenate. From inspection of their data it seems that 3 to 7 Gm. of the salt would usually kill a chicken, although they they did not state such a conclusion.

McCulloch (2) determined that lead arsenate equivalent to 2 Gm. of arsenic trioxide is required to kill a sheep weighing approximately 40 Kg.

The M. L. D. for rabbits and hares is 200 mg./Kg., according to Chappelier (3). Apparently no other work has been recorded for the acute toxicity of lead arsenate for higher animals.

EXPERIMENTAL

The lead arsenate used had the formula $PbHAsO_4$ and was 200 mesh or less in fineness.

The toxicity was expressed as the LD_{50}, which was calculated by the method of Behrens as described by Burn (4). The rationale of this method was originally proposed by Dragstedt and Lang (5).

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† Abstract of a part of a dissertation presented to the Graduate Council, University of Florida, by J. L. Voigt, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Chickens.—White Leghorn roosters, approximately 2 Kg. in weight, were force fed with the calculated single dose in a capsule. The first symptom of poisoning was a lack of appetite. Upon autopsy of the dead birds it was seen that the crop and proventriculus were inflamed, the gizzard was necrotic, and the small intestine inflamed and hemorrhagic.

Doses of 500 to 5000 mg./Kg. were given to a series of ten birds. All but two birds died within forty days, the survivors having been fed 1000 and 1500 mg./Kg., respectively.

Following these exploratory doses ten chickens were given 1000 mg./Kg. of lead arsenate with ten deaths occurring in seven to fourteen days.

Another ten birds were given 500 mg./Kg. of the compound. Each of these died in eight to eighteen days. The loss in weight and the red cell count were determined on the birds still alive after twelve days with following results:

TABLE I.—RED CELL COUNT AND CHANGE IN WEIGHT IN CHICKENS AFTER FEEDING 500 MG./KG. OF LEAD ARSENATE

Weight When Fed (3/8/45), Kg.	Weight 12 Days Later (3/20/45), Kg.	Red Cell Count	Date of Death
2.23	1.47	1,860,000	3/20/45
2.16	1.36	2,450,000	3/26/45
1.80	1.35	1,600,000	3/23/45
1.72	1.28	1,780,000	3/21/45

The average red cell count for chickens is considered by Scarborough (6) to be 3,440,000, with a normal range of 2,800,000 to 4,500,000.

Ten chickens were given 300 mg./Kg. of lead arsenate. All survived. After thirty-three days the red cell count was only slightly below normal and the weight loss was small.

To another ten chickens 400 mg./Kg. were administered. Three deaths occurred in twelve days while the survivors showed no consistent change in weight or red cell count.

The final ten chickens were given 450 mg./Kg. with four deaths resulting in twelve days. There was some loss in weight among the survivors. By calculation from the data given in Table II it was found that the LD_{50} for chickens is near 450 mg./Kg.

White Rats.—An aqueous suspension of lead arsenate was fed by stomach tube to white rats of both sexes, most of whom weighed between 150 and

TABLE II.—MORTALITY FOR CALCULATING THE LD_{50} OF LEAD ARSENATE IN CHICKENS

Dose, Mg./Kg.	Observed Mortality	Deduced Mortality
1000	10/10	..
500	10/10	..
450	4/10	7/13
400	3/10	3/16
300	0/10	..

230 Gm. An exploratory test was carried out by feeding ten rats doses varying from 175 to 425 mg./Kg. No toxic symptoms developed.

Ten rats, given 500 mg./Kg., exhibited diarrhea and lack of appetite for a few days; one death occurred after twenty-five days. In another group, ten rats were given 600 mg./Kg. with two deaths resulting within three weeks. A dose of 700 mg./Kg. caused three deaths in ten animals in fifteen to twenty days; upon examination of the dead rats symptoms of irritation of the intestinal tract were observed. In another group four rats out of ten died from 800 mg./Kg. within six days; the others survived. In the next series of ten rats which received 900 mg./Kg., five deaths occurred in six to eighteen days. The final group received 1000 mg./Kg. and seven out of ten died in two to twelve days. All the dead rats showed similar indications of arsenic poisoning on post-mortem examination. The LD_{50} for lead arsenate in white rats was calculated from the data given in Table III as approximately 825 mg./Kg.

TABLE III.—MORTALITY FOR CALCULATING THE LD_{50} OF LEAD ARSENATE IN WHITE RATS

Dose, Mg./Kg.	Observed Mortality	Deduced Mortality
1000	7/10	22/25
900	5/10	15/23
800	4/10	10/24
700	3/10	6/27
600	2/10	3/32
500	1/10	1/39

Rabbits.—The lead arsenate was administered to rabbits of both sexes by stomach tube in the same way as it was given to the white rats. These animals were obtained from various sources and weighed between 1 and 2 Kg.

Ten rabbits were given doses varying between 100 and 325 mg./Kg. with the result that all died within six days. Post-mortem examinations showed the characteristic picture of lead arsenate poisoning: inflammation, necrosis, and clotting of blood in the gastrointestinal tract.

Eight more rabbits were given doses of 5 to 100 mg./Kg. The animals receiving 25, 50, and 100 mg./Kg. died, while the one receiving 75 mg./Kg. eventually recovered after exhibiting toxic symptoms.

After these preliminary trials ten rabbits were fed 20 mg./Kg.; one animal died ten days later. The next group of ten animals received 40 mg./Kg.; four days later one rabbit died. Fifty mg./Kg. were fed to each of seven animals in another group, with one death being recorded four days later. Sixteen rabbits were given 100 mg./Kg. with six deaths within three days. Ten more rabbits were fed 150 mg./Kg. and four died in four to twelve days. In the final series ten rabbits received 200 mg./Kg. All died within two days with severe necrosis and profuse bleeding in the stomach. The LD_{50} for lead arsenate in rabbits was calculated as approximately 125 mg./Kg. from the data in Table IV.

TABLE IV.—MORTALITY FOR CALCULATING THE LD_{50} OF LEAD ARSENATE IN RABBITS

Dose, Mg./Kg.	Observed Mortality	Deduced Mortality
200	10/10	...
150	4/10	13/19
100	6/16	9/25
50	1/7	3/25
40	1/10	2/33
20	1/10	1/41

SUMMARY

1. From the results of feeding lead arsenate to 50 chickens in doses varying from 300 to 1000 mg./Kg. it was calculated that the LD_{50} is near 450 mg./Kg. for these fowl. All chickens receiving doses of 500 and 1000 mg./Kg. died. This dose compares with the fatal dose of 60 to 150 mg./Kg. for arsenic trioxide in chickens as tabulated by Heffter (7).

2. The weight and the red cell count of chickens decreased after ingestion of 500 mg./Kg. of lead arsenate.

3. The LD_{50} for lead arsenate in white rats was calculated as approximately 825 mg./Kg. after feeding doses of 500 to 1000 mg./Kg. to 60 animals. After the largest dose seven out of ten animals died.

4. Doses of 20 to 200 mg./Kg. of lead arsenate were fed to 63 rabbits and the LD_{50} calculated therefrom as about 125 mg./Kg. A dose of 200 mg./Kg. killed ten rabbits out of ten. Arsenic trioxide has been reported as fatal by Heffter (7) in doses of 10 to 30 mg./Kg.

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The Action of Intestinal Enzymes upon Cellulose Acetate Phthalate and Butyl Stearate Enteric-Coated Tablets*†

By CHARLES W. BAUER‡ and PETER E. MASUCCI

In considering the disintegration of enteric coatings of tablets it is essential to differentiate between intestinal contents and intestinal secretions. Enteric-coated tablets disintegrate in the intestinal contents which are seldom, if ever, alkaline. The disintegration of cellulose acetate phthalate and butyl stearate is due to the hydrolytic effect of the intestinal esterases and not, as is commonly believed, upon the alkalinity of the intestines.

IT IS generally agreed that more and more medicines should be enteric coated, both for the avoidance of gastric distress and for the assurance of therapeutic action. The preparation of a satisfactory enteric coating is not a simple procedure, nor is its evaluation a simple and easy task. Unfortunately many of the enteric coatings now on the market have not been properly evaluated. It seems, then, that if we are going to be called on for additional enteric-coated preparations, further work in this field of investigation is desirable.

The demands, as well as the restrictions, that pharmacy has placed upon the materials that are used to make enteric preparations are fairly well known. Very briefly, the specifications call for a nontoxic and physiologically inert material that must withstand the action of the gastric juice, but must disintegrate in the intestines.

The criticism that should be made about enteric preparations is directed against the tests that are used to evaluate them. It is assumed that the coating that is used is nontoxic and is physiologically inert, for these qualifications can be sufficiently tested. Attention should be devoted to the other tests, especially to those that involve the use of acids and alkalies. A typical report on the analysis of an enteric coating states that it is resistant to 0.3 per cent hydrochloric acid at body temperature for four and one-half hours, and that it is completely broken down in a 0.5 per cent sodium bicarbonate solution in one and one-half hours. On the strength of this analysis the substance is said to be satisfactory. Literature citations and experimental evidence presented in this paper point out that the effectiveness of an enteric coating cannot be evaluated upon its disintegration in a solution of sodium bicarbonate.

Certain coatings already known to be unsatisfactory were not studied in this work. Keratin was tested by Bukey and Rhodes (1), and was found to be highly efficient when properly applied, but very difficult of proper and even application. Formaldehyde-gelatin was tested by Goorley and Lee (2), who found it undesirable because upon aging it became too hard and would not disintegrate. Bukey and Brew (3) found tolu to be of very little value because it, too, would fail to disintegrate if not freshly made. Worton, Kempf, Burrin, and Bibbins (4) found collodion to be of no use at all because of its insolubility in the intestinal juices. These authors found agar and elm bark also to be unsatisfactory, because the time of disintegration was too variable.

Cellulose acetate phthalate has received considerable publicity as a very satisfactory enteric coating. The literature (5) indicates that this substance does not disintegrate in the stomach but does disintegrate in the small intestine. However, the reasons given for the decomposition of cellulose acetate phthalate seem to be erroneous. It is true

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that it does not disintegrate in 0.3 per cent hydrochloric acid in four hours and does disintegrate in a 0.5 per cent sodium bicarbonate solution in one-half hour. But its behavior in such *in vitro* tests cannot be assumed to explain its action *in vivo*.

The intestinal juice does not resemble a 0.5 per cent solution of sodium bicarbonate. Then why is this test made? It is made because of a widespread erroneous belief that the contents of the intestines are alkaline. This error has crept into pharmaceutical and medical literature and will require some time to be corrected. There is ample evidence to show that the contents of the intestines must be distinguished from the secretions of the intestines, and are seldom, if ever, alkaline. McClendon (6) has shown that the contents of the intestines are acid, the *p*H rising in the distal levels, but even in the ileum the contents are rarely above neutral. A good review of this subject has been given by Verzar (7).

was followed with this mixture as has been described for that in flask 1.

In flask 3, 1 cc. of a 2% solution of calcium chloride, 1 Gm. of Pancreatin U. S. P., and 25 cc. of distilled water were added and were brought to the boiling point to destroy the enzymes in the Pancreatin. The mixture was then cooled to room temperature and 1 Gm. of cellulose acetate phthalate was added. This mixture was then placed in a constant temperature bath at 37° for one hour and was then titrated with 0.1 *N* sodium hydroxide as above.

Into flask 4, 1 cc. of a 2% solution of calcium chloride, 1 Gm. of cellulose acetate phthalate, 1 Gm. of Pancreatin U. S. P., and 25 cc. of distilled water were placed. The same procedure was carried out here as with flasks 1 and 2. The titration showed that flask 4 required more 0.1 *N* sodium hydroxide than was required in the other three flasks. This seems to indicate that the cellulose acetate phthalate was acted upon by the pancreatic esterases. The hydrolytic effect of the esterases upon the cellulose acetate phthalate produced acids, which accounts for the use of a greater amount of 0.1 *N* sodium hydroxide for neutralization. The experimental evidence of these findings may be seen in Table I.

In the next step of the investigation, cellulose

TABLE I.—EFFECT OF PANCREATIC ESTERASE ON CELLULOSE ACETATE PHTHALATE AT 37° FOR ONE HOUR SHOWN BY THE QUANTITY OF 0.1 *N* SODIUM HYDROXIDE TO NEUTRALIZE THE REACTION MIXTURE

	Flask 1 (Control)	Flask 2 (Control)	Flask 3 (Control)	Flask 4
Calcium chloride 2%, cc.	1	1	1	1
Cellulose acetate phthalate, Gm.	1	..	1	1
Pancreatin U. S. P., Gm.	..	1	1 (boiled)	1
Distilled water, cc.	25	25	25	25
0.1 *N* sodium hydroxide, cc.	11.2	6.8	18.5	28.1

EXPERIMENTAL

Cellulose Acetate Phthalate as an Enteric Coating.—Cellulose acetate phthalate is an organic ester that should be acted upon by the esterases. To determine the effect of esterases upon cellulose acetate phthalate experiments were made in which Pancreatin U. S. P. was used as the source of esterases for this determination.

Four Erlenmeyer flasks of 125-cc. capacity were designated as flask 1, flask 2, flask 3, and flask 4. Into flask 1, 1 cc. of a 2% solution of calcium chloride, 1 Gm. of cellulose acetate phthalate, and 25 cc. of distilled water were placed. This flask was placed in a constant temperature bath maintained at 37° for one hour. After one hour the mixture was titrated with 0.1 *N* sodium hydroxide using thymol blue as the indicator.

Into flask 2, 1 cc. of a 2% solution of calcium chloride, 1 Gm. of Pancreatin U. S. P., and 25 cc. of distilled water were placed. The same procedure

acetate phthalate was applied as an enteric coating to 5-gr. sodium salicylate tablets. The formula for the cellulose acetate phthalate mixture found most satisfactory was introduced by Abbot and Allport (8). This formula is as follows:

Cellulose Acetate Phthalate.....	5.0 parts
Ethyl Acetate.....	47.5 parts
Alcohol 95%.....	47.5 parts

A satisfactory method of coating these tablets was to place them in a revolving copper drum and to apply the coating in the form of a fine spray from an atomizer. At the same time a blast of hot air was directed into the drum to hasten the evaporation of the solvent and the drying of the coating. After the tablets had been well coated, they were weighed to determine how much coating was on each tablet. The coating was found to average 0.4 gr. on each tablet. The test used to determine the quality of the coating consisted in allowing the tablets to stand in a dilute solution of ferric chloride for two or

three hours. If the coating contained "pinholes" the sodium salicylate would soon manifest itself by a dark coloration of the solution. If the tablets withstood this test, they were considered to be satisfactory for the next step in the investigation.

The next step was to submit the tablets to *in vitro* analysis not only in the manner in which it is ordinarily done, but also in a new test, based upon the fact that the intestinal contents are neutral or even acid. The following solutions were prepared for this part of the analysis.

1. *Artificial gastric juice* (*9*):

Pepsin N. F.	0.5 Gm.
Hydrochloric Acid	0.7 Gm.
Lactic Acid	0.2 Gm.
Distilled Water	100.0 cc.

2. *Artificial intestinal juice* (*older type*) (*10*):

Pancreatin U. S. P.	0.1 Gm.
Sodium Bicarbonate	1.0 Gm.
Distilled Water	100.0 cc.

3. *Artificial intestinal juice* (*newer type*) (*11*):

Pancreatin U. S. P.	1.0 Gm.
Calcium Chloride Solution (1%)	1.0 cc.
M/5 Potassium Biphosphate	25.0 cc.
M/5 Sodium Hydroxide	11.8 cc.
Distilled Water	100.0 cc.

These solutions were maintained at 37° by means of a constant temperature bath. Five coated tablets were placed in the artificial gastric juice to determine the length of time it would take for the gastric juice to act upon the coating. The coated tablets not only remained intact for four and one-half hours but showed no signs of disintegration in seven days. The artificial gastric juice was kept in motion by a motor-driven stirrer during the entire period of observation.

Five of the coated tablets were placed in the artificial intestinal juice (older type) at a *p*H of 8.5. This mixture also was kept at a constant temperature of 37°; and it, too, was stirred mechanically. All five tablets disintegrated within fifty minutes.

Finally five of the coated tablets were placed in the artificial intestinal juice (newer type). This juice was prepared in accordance with the new theory that the contents of the intestines are not alkaline. It had a *p*H of 6.9. The same conditions prevailed here as with the other two solutions; that is, the temperature was maintained at 37°, and continual motion was obtained by means of a mechanical stirrer. Once again the length of time was noted for disintegration. All five tablets first showed signs of rupture after from seventy to seventy-five minutes. Thus it was seen that the enteric-coated tablets stood up remarkably well in the artificial gastric juice but were acted upon by both types of artificial intestinal juice. The newer type of artificial intestinal juice, even though it was started at a *p*H of 6.9 and certainly did not become more alkaline as the digestion took place, brought on disintegration of the tablets because of the enzyme action, and not because of the alkalinity of the solution. Repeated trials gave similar results. It was further observed that a 1% solution of sodium bicarbonate without any Pancreatin caused the tablets to disintegrate in one hour, while the buffered solution without the Pancreatin had no effect upon the tablets.

Since the enteric coating of cellulose acetate phthalate was seen to be acted upon by pancreatic esterases when the test was made *in vitro*, the next step was to determine how it would act *in vivo*. Sodium salicylate was the drug chosen for this purpose since it has been shown by Lazenski and Diver (12) that this drug is readily detected in the urine by ferric chloride. The absence of a reaction indicates that no disintegration of enteric-coated sodium salicylate has taken place. It is known that 5 drops of a 1% aqueous solution of sodium salicylate gives, with ten drops of 10% solution of ferric chloride, a violet color which is permanent for more than fifteen minutes. Now if a tablet of sodium salicylate is enteric coated and is then swallowed, there will be no sodium salicylate found in the urine until the coating has disintegrated and the salicylate has been absorbed and has reached the urine. If a positive test is obtained in less than two or three hours, it probably indicates that the enteric coating has been acted upon in the stomach. However, if a positive test is not obtained until five or six hours after the tablet has been ingested, it usually indicates that the coating has withstood the action of the gastric juice and has disintegrated in the intestine. If no test at all is obtained, then the coating has not disintegrated and the tablet is usually found in the feces intact.

Two 5-gr. tablets of sodium salicylate coated with cellulose acetate phthalate were taken orally with two tumblerfuls of water. The time of administration was recorded, and at hourly intervals the urine was tested for salicylates in the manner described. For controls a group of normal adults were given two 5-gr. tablets of sodium salicylate. The bladder was emptied before the tablets were taken and samples of urine were then assayed at hourly intervals. In 5 subjects a positive test was obtained in one hour while in one subject the test was only moderately positive in one hour but was very strong at the end of two hours. It will be noted that the cellulose acetate phthalate tablets did not give a positive test in less than four hours. It will also be observed that the majority of the subjects gave positive tests in a period of six hours. Hence, it may be assumed that the cellulose acetate phthalate readily disintegrates in the small intestine. Those tablets requiring more than seven hours for a positive test may have reached the colon before suffering disintegration. Since cellulose acetate phthalate is acted upon by pancreatic esterases, and since the intestinal contents are not alkaline, it appears that this ester suffers disintegration in the intestines because it serves as a substrate for intestinal esterases. The experimental results are summarized in Table II.

TABLE II.—TIME REQUIRED FOR SODIUM SALICYLATE TO APPEAR IN THE URINE WHEN TWO CELLULOSE ACETATE PHTHALATE ENTERIC-COATED SODIUM SALICYLATE TABLETS WERE INGESTED

No. of Adult Subjects	No. Hours Required for a Positive Test									
	1	2	3	4	5	6	7	8	9	10
5 uncoated tablets	+	..	..	..	..	..	..	..	..	..
1 uncoated tablet	−	+	..	..	..	..	..	..	..	..
1	−	−	−	+	..	..	..	..	..	..
2	−	−	−	−	+	..	..	..	..	..
18	−	−	−	−	−	+	..	..	..	..
2	−	−	−	−	−	−	+	..	..	..
2	−	−	−	−	−	−	−	+	..	..
1	−	−	−	−	−	−	−	−	+	..
1	−	−	−	−	−	−	−	−	−	+

TABLE III.—TIME REQUIRED FOR SODIUM SALICYLATE TO APPEAR IN THE URINE WHEN TWO BUTYL STEARATE ENTERIC-COATED 5-GR. SODIUM SALICYLATE TABLETS WERE INGESTED

No. of Subjects	No. Hours Required for a Positive Test								
	1	2	3	4	5	6	7	8	9
5 uncoated tablets	+	..	..	..	..	..	..	..	..
1	−	−	−	−	+	..	..	..	..
6	−	−	−	−	−	+	..	..	..
5	−	−	−	−	−	−	+	..	..
4	−	−	−	−	−	−	−	+	..
4	−	−	−	−	−	−	−	−	+

Butyl Stearate as an Enteric Coating.—The ester, butyl stearate, when used in the same manner as cellulose acetate phthalate, as an enteric coating for sodium salicylate tablets, required a longer period of time for the salicylate to appear in the urine. One reason for this longer period of time was undoubtedly due to the fact that twice as much butyl stearate than cellulose acetate phthalate had to be applied in order to get an impervious coating. A further study of the effectiveness of butyl stearate as an enteric coating was made by applying it to barium sulfate tablets and by observing the place of distingegration by means of X-ray visualization.

Five-grain tablets of sodium salicylate as well as 5-gr. tablets of barium sulfate were coated with the following mixture:

Butyl Stearate	50 parts
Shellac	100 parts
Alcohol	200 parts

The tablets were placed in a revolving drum; the butyl stearate mixture was applied from a beaker; talc was used as the running powder, and air was directed on the revolving tablets to help dry the coating.

The increase in weight of each barium sulfate tablet was 0.8 gr. Only one barium sulfate enteric-coated tablet was given to each subject.

It was found that the barium sulfate enteric-coated tablets passed through the jejunum before they uffered disintegration. About 50% of the trials showed disintegration to take place in the lower ileum and about an equal number reached the caecum; but not all of the tablets that reached the caecum disintegrated within eight hours. It was impractical to hold the subjects for a longer period of time. While most drugs are not absorbed from the large intestine it can be shown that sodium salicylate will give a urine test when administered rectally. Hence, some of the positive tests one may obtain when sodium salicylate is used to test enteric coatings fail to give the position where the distingegration takes place. It seems that butyl stearate may not serve as a useful enteric coating when it is applied in the manner indicated in this paper, if the drug on which it is applied is intended for the circulation; it may, however, be useful if the drug is not intended for the circulation but is used for its action upon organisms in the lower ileum or colon.

In all cases the tablet was given within one hour after breakfast. The majority of the tablets moved out of the stomach in less than two hours. All sub-

TABLE IV.—TIME REQUIRED FOR ONE BARIUM SULFATE TABLET TO DISINTEGRATE IN THE INTESTINAL TRACT WHEN COATED WITH BUTYL STEARATE AS SEEN BY X-RAY VISUALIZATION

No. of Subjects	Place of Disintegration	No. Hours After Ingestion				
		4	5	6	7	8
2	Ileum	−	+	..	..	..
3	Ileum	−	−	+	..	..
5	Ileum	−	−	−	+	..
2	Colon	−	−	−	−	+
5	In colon but no disintegration	−	−	−	−	−
1	In stomach but no disintegration	−	−	−	−	−

jects had a luncheon before the tests were completed.

The results obtained with butyl stearate enteric-coated sodium salicylate tablets may be seen in Table III.

The results obtained with butyl stearate enteric-coated barium sulfate tablets observed by means of X-ray visualization may be seen in Table IV.

SUMMARY

1. One interested in the disintegration of enteric coatings must differentiate between intestinal secretions and intestinal contents.

2. Enteric-coated tablets disintegrate in the intestinal contents, and not in the intestinal secretions.

3. The *p*H of the intestinal contents is seldom, if ever, alkaline.

4. The disintegration of enteric coatings like cellulose acetate phthalate in the intestinal contents, which are on the acid side, is the result of the hydrolytic action of the intestinal esterases.

5. Cellulose acetate phthalate is a satisfactory enteric coating that for the most part disintegrates in the jejunum.

6. Butyl stearate seems not to disintegrate in the jejunum as seen by X-ray visualization, but does show disintegration in the ileum and colon. This slowness in disintegration may be due to the fact that a heavier coating of butyl stearate than cellulose acetate phthalate was used. Some of the butyl stearate enteric-coated tablets failed to disintegrate in eight hours.

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Book Reviews

Pharmacology and Pharmacotherapeutics for Dentists. Third Edition. By WILLIAM H. O. MCGEHEE and MELVIN W. GREEN. The Blakiston Company, Philadelphia, Pa., 1947. xx + 536 pp. 16 x 23.5 cm. Price $6.50.

This excellent pharmacology textbook for dental students has been extensively revised for this edition. The authors have recognized the rapid development of new drugs and the changes in our knowledge of various drug actions. Among the parts brought up to date, especially from the viewpoint of dental practice, are those on the sulfonamides, vitamins, hormones, and antibiotics.

The book is divided into eight sections, each covering a particular part of the general field. The first section is an introduction to the basic principles of pharmacology, pharmacy, and prescription writing. The other sections cover the locally acting drugs, especially those used in the mouth or on the teeth, the systemically acting drugs, and the miscellaneous substances such as the metals, specifics, vitamins, and hormones. The appendix includes sections on the symptoms and treatment of acute poisoning, various useful tables, and a comprehensive dental formulary.

While the authors have covered the entire field of pharmacology, they have emphasized the drugs useful to the dentist. The drugs which are considered, in greatest detail include antiseptics, disinfectants, irritants, caustics, bleaching agents, dentifrices, local anesthetics, central stimulants, anesthetics, and analgesics.

Some of the material appears to be superfluous from the standpoint of the dental student. For example, the section on the theories of drug action is too short to be of value and just long enough to be confusing to the student. The systemic effects of procaine do not appear to be adequately presented, especially in view of the wide usage of this drug. The statement that the action of ephedrine is to inhibit epinephrine oxidase is not correct.

This book is well written and should be very useful both to the student and to the practicing dentist.—RAYMOND P. AHLQUIST.

Estimation of the Vitamins. Edited by W. J. DANN and G. HOWARD SATTERFIELD. The Jaques Cattell Press, Lancaster, Pa., 1947. 531 pp. 16 x 25 cm. Price $6.50.

Methods of Vitamin Assay. Prepared and edited by the ASSOCIATION OF VITAMIN CHEMISTS. Interscience Publishers, Inc., New York, 1947. xviii + 189 pp. 15 x 23 cm. Price $3.50.

The biological assay of the vitamins is as old as knowledge of their existence. The isolation and chemical characterization of the vitamins stimulated publication in this important assay field so that during the past fifteen years thousands of publications have appeared. Because complex foodstuffs are the source of the vitamins, their separation and measurement are not always easy. The problem is further complicated by the fact that many of the vitamins occur in nature in more than one chemical state. Vitamin A often occurs as esters. Carotene has vitamin A activity, but there is more than one carotene and they do not have equal physiological activity. Thiamin can occur as co-carboxylase; nicotinic acid can also exist as the amide. Ascorbic acid sometimes is present as dehydro-ascorbic acid—and so on it goes.

Vitamins may be assayed biologically by preventive and curative techniques. They may be determined by a number of chemical techniques—colorimetric methods, ultraviolet light absorption methods, simple titrations, gasometric methods and others. In addition they are often determined by microbiological methods. Even a human biological assay, based in part on chemical methods, has been proposed.

Confronted with the myriad of papers in the literature, many of them dealing with specific methods for special purposes, the average worker entering the field is almost hopelessly confused. To coordinate the literature and standardize the vitamin methods is the purpose of these two useful volumes.

The first of these volumes, Volume XII of the Biological Symposia series, represents the review type approach. Each of the 29 chapters is written by an expert. E. M. Nelson, Esmond E. Snell, C. A. Elvehjem, John V. Scudi, Charles E. Bills, H. J. Almquist, and many other such famous names grace the pages of this book. In general, separate chapters are written on bioassay, physico-chemical methods, and microbiological methods for each of the vitamins. All of the well-characterized vitamins are included. Each chapter contains its bibliography.

The second book is quite unique and is more of a manual than a review treatise. It was prepared under the jurisdiction of the Association of Vitamin Chemists. Only such methods have been included which are in general use as determined by committees of specialists. Emphasis has been placed on those details on which the usefulness and precision of a method depend. Extensive notes have been added. Only where more or less essential are literature references cited. These two books are natural complements to each other and certainly they will soon become the "bibles" of workers in the vitamin field.

The Water-Soluble Gums, by C. L. MANTELL. Reinhold Publishing Corporation, 330 W. 42nd St., New York 18, N. Y. v + 279 pp. 15 x 23 cm. Price $6.

For many years practice and art were ahead of science in the gum field; however, the author has in this book attempted to coordinate the information relating to the water-dispersible products derived from a wide variety of plants and known as gums. In this he has succeeded very well because he has brought together into 16 chapters all of the well-known, and not so well-known, gums and has classified them into groups with similar properties.

In each of these groups of gums the origin, physical, and chemical properties are discussed thoroughly. The author also includes an excellent chapter on Specifications, Identification, and Testing of all of the more well-known gums giving description, physical properties, tests for identity and purity, and 37 methods for the testing of the various constituents of the gums.

Also included is an extensive table of the Nature Designation of the Gums, consisting of name, place of origin, type, and present-day classification; a botanical index giving the botanical name and page reference of all gums discussed in the book; and an index of all authors cited in references.

This book is well written, interesting, and technical only to the point of necessity and should be of value to anyone interested or working in the field of "gum technology."

Fatty Acids: Their Chemistry and Physical Properties, by KLARE S. MARKLEY. Interscience Publishers, Inc., New York, 1947. x + 668 pp. 15 x 23 cm. Price $10.

The author of this excellent treatise has brought together the essential literature on the fatty acids in an organized fashion. The book is divided into six sections containing a total of 23 chapters. The sections are entitled: The Nature and History of Fats and Waxes, Classification and Structure of Fatty Acids, Physical Properties of the Fatty Acids, Chemical Reactions of the Fatty Acids, Syntheses of Fatty Acids, and Isolation of Fatty Acids.

The work is thoroughly modern in its outlook with considerable of the physical-chemical approach. Careful and discriminating documentation can be seen throughout. The author shows evidence of having tried to select the most correct physical properties from data which often extend back over about fifty years and frequently covered periods when the methodology was not so advanced.

Fatty acids play such an important role in pharmacy, that no research-minded pharmaceutical scientist should be without this reference.

Encyclopedia of Chemical Technology. Volume I. Edited by RAYMOND E. KIRK and DONALD F. OTHMER. In 10 volumes. The Interscience Encyclopedia, Inc., New York, 1947. xxiv + 981 pp. 18 x 26.5 cm. Price $20.

Good technical encyclopedic works should be the foundation of a technical library. While such references have limitations they constitute a starting point for gleaning information on a particular subject. Encyclopedias in current use often leave the reader with a sense of inadequacy, however, the revisions usually have not been sufficiently modern nor thorough. Frequently references to the primary source are ancient or even missing. That is why libraries will welcome the new *Encyclopedia of Chemical Technology.*

The contributors to this first volume are well-recognized authorities in their fields. Such men as H. H. Anderson and M. S. Dunn of the University of California; P. H. Emmett of the Mellon Institute; W. H. Hartung and John C. Krantz, Jr., of the University of Maryland; E. B. Sandell of the University of Minnesota; and Louis Schwartz of the National Institute of Health, have made valuable contributions. In addition, many research and development workers in such industrial establishments as E. I. du Pont de Nemours and Company, Dow Chemical Company, The American Cyanamid Company, General Aniline and Film Company, Aluminum Company of America, Sterling Drug Company, Rohm and Haas, Schenley Distillers Corporation, Monsanto Chemical Company, and Carbide and Carbon Chemicals Company are represented. In most instances the reader finds himself saying, "He is the very person to write that section."

Volume I constitutes the A's up to Anthrimides and embraces such subjects as acetanilid, acid-base systems, absorption, agar, air conditioning, alcohol, algin, alkaloids, allergens, amebacides, alkylation, amines, and antacids.

Typical of an individual compound or product is the section on algin. After a brief introductory account of origin, trade names, and commercial forms, there appears a brief account of the chemical nature of algin and its properties and reactions. A section on sources and production follows. The early processes of manufacture are briefly described and then the Green cold process and the LeGloahec-Herter process are described in considerable detail, amplified by flow sheet diagrams.

The uses of algin are given in about one and a half pages. However, under each significant use is a cross-reference to another volume, i.e., the reader can expect greater detail on dental uses, pharmaceutical uses, cosmetic uses, and uses in textile finishing elsewhere. A bibliography of 12 references follows. The section was prepared by Dr. C. K. Tseng of the Scripps Institution of Oceanography. This section is typical of those describing individual compounds or products excepting that in some cases more detail concerning toxicology and economics is given.

The section on amines, written by Dr. E. F. Landau, is representative of articles written about chemical classes. After a brief discussion of classes of amines, he discusses nomenclature and then general properties. A section on aliphatic amines with a tabulation of the properties of 39 monamines, 9 di- and polyamines and 2 alicyclic amines follows. The subsection on aromatic amines includes a tabulation of 50 aromatic amines. In all cases the preparation, occurrence, and physical and chemical properties are given.

A number of general drug divisions classified by pharmacological activity are included. The section on amebacides written by H. H. Anderson and Eder L. Hansen is representative. The authors outline the requirements of a good amebacide and describe Leake's method for testing for amebacidal activity. The value of *in vitro* vs. *in vivo* screening is discussed. This is followed by a tabulation of 72 drugs which have been shown to have some degree of amebacidal activity and a table of the cysticidal activity of 20, cationic detergents. Similar sections on allergens, analgesics, anesthetics, and antacids occur in this volume.

It is planned that two to three volumes will be issued per year until the entire encyclopedia of ten volumes is completed.

The binding is in black buckram with gold letters on a medium blue trim. The paper is of excellent quality and the typography is good.

When the series is complete, it will be an exceptionally useful addition to any technical library.

The Chemistry and Technology of Waxes, by ALBIN H. WARTH. Reinhold Publishing Corporation, 330 W. 42nd St., New York 18, N. Y. viii + 519 pp. 15 x 23 cm. Price $10.

Here is a book well worth having as a ready reference for anyone who is interested in the technology of waxes. The author has brought together and correlated a mass of widely scattered information about waxes and has molded it into a logical arrangement of facts according to source, chemical and physical properties, and uses of the waxes.

A particularly good and lengthy discussion of the "Natural Waxes" is included. This is followed by more brief discussions of Fossil and Earth Waxes, and Lignite Paraffins; Petroleum Waxes; Synthetic Waxes and Wax Compounds; Emulsifiable Waxes, Waxy Acids and Metallic Soaps; Methods for Determining the Constants of Waxes; and Uses of Waxes in Industry. The chapter on Methods for Determining the Constants of Waxes should be of special interest to the analytical chemist.

Also of special interest are many tables on the physical constants of waxes which are included in the appendix together with an author index. Many references have been included at the end of each chapter, by the author, to provide further information on the subjects discussed.

A Manual of Pharmacology and Its Applications to Therapeutics and Toxicology, by TORALD SOLLMANN. 7th Edition, 1948. W. B. Saunders Company, Philadelphia. ix + 1132 pp. 17.5 x 26 cm. Price $11.50.

To some there is magic in the number 7, but we suspect that "midnight oil," judgment based upon years of experience in the field, and a knowledge of the needs of the physician, all mixed *secundum artem* are more responsible for the outstanding character of the 7th edition of this manual, than magic.

Users of previous editions who miss the large number of references to the older literature will be compensated by the care with which references were selected. Gone are most of the references to the wishful and in their place one finds the truthful and the hopeful.

Readers desiring up-to-date information on the sulfonamides, the antibiotics, the antihistaminics, antithyroid drugs, folic acid, "nitrogen mustards," and other new drugs, will find it here with as much detail as the present status warrants.

During recent years the pendulum of therapeutics has swung again toward the use of more "heroic" doses. The use of larger doses of highly potent drugs demands more detailed knowledge of such factors as mode of drug action, essential elimination rates, blood levels, toxic and untoward signs. Dr. Sollman has kept these factors in mind and has covered them very well.

Students and other readers alike will appreciate the more readable style and format used in this edition.

Pharmacology, Therapeutics and Prescription Writing, by WALTER A. BASTEDO. 5th Edition, 1947. W. B. Saunders and Company, Philadelphia. x + 840 pp. 15 x 23 cm. Price $8.50.

The publication of the 5th edition of any book by a prominent and capable author scarcely calls for a review since its very existence is mute testimony to its value.

In the 5th edition, the author has added such "new" drugs as amino acids, rutin, the blood plasma fractions of Cohn, antihistamines, anticonvulsants, folic acid, demerol, metopon, BAL, and streptomycin.

Although written especially for the practicing physician, this text appeals strongly to pharmacists and pharmacy students. The author has been careful to describe whatever physiology is needed for the understanding of the action of any drug group. This is especially helpful, for even medical students can forgot numerous details of physiology.

Possibly pharmacists are hypercritical about a matter which is of less consequence in a book on pharmacology when they insist on the accurate accreditment of drugs to the U. S. P. or N. F. Time and again we have noted drugs which have been in the N F. for as long as two revisions still accredited to N. N. R. by textbook writers. Drugs which have long ago been changed from U. S. P. to N. F. and vice versa, are often incorrectly listed. The reader of such books is entitled to know with accuracy to which official text to turn for further information. Dr. Bastedo is one of the few writers who appears to be conscious of this deficiency and very few such errors can be found in his book. The reviewer believes that Dr. Bastedo is supplying a genuine service in evaluating the official status of drugs with such care.

Cornell Conferences on Therapy, Volume II. Edited by HARRY GOLD, DAVID P. BARR, MCKEEN CATTELL, EUGENE F. DUBOIS, PAUL A. BUNN, AND WALTER MODELL. New York, 1947, Macmillan Company. xx + 354 pp. 13 x 20.5 cm. Price $3.75.

How would you like to sit in on a conference on therapy with the most eminent pharmacologists and clinicians of Cornell University Medical School? Do you believe Iron, Quinine and Strychnine Elixir is a placebo? What modern therapeutic uses do placebos have? What is the present treatment of meningitis? Is the new short treatment of venereal disease successful? What is the status of oral penicillin? What are the comparative actions of the commonly used sympathomimetic amines? Is the use of skin antiseptics in surgery of genuine value or is it merely a ritual?

This book gives you an opportunity to find the answers to these questions and many more of equivalent significance. The material found in the book is an edited transcription of the Conferences on Therapy held periodically during the academic year at Cornell University Medical School. The discussions are informal but with direction. Naturally they represent diverse opinions and experiences. No attempt is made to treat subjects exhaustively, but rather to wed the practical approach of clinical medicine to the theory as discovered and interpreted in pharmacological science. In short, it is a teaching dodge to try to find a meeting ground for the practical and the theoretical.

While written for the physician, the pharmacist will find much background material here and suggestions occasionally for points of professional contact with the physician.

Note

Viburnum Studies. XIV. A Note on the Variability of Potency of Commercial Viburnum Preparations*

By A. B. SLOANE, A. R. LATVEN, and JAMES C. MUNCH

IN CONNECTION with our studies on Viburnum, methods of bioassay were proposed in an earlier report (1). At that time we were using the isolated guinea-pig uterus, measuring antagonism to U. S. P. Standard Powdered Posterior Pituitary; also neutralizing the depressor action of Viburnum on anesthetized dogs with U. S. P. Reference Standard Epinephrine. Further research indicated certain advantages in the use of the isolated uterus of the rat, and comparison of the depressor potency of Viburnum with the depressor potency of papaverine HCl on the same uterine strip (2).

Using the isolated rat uterine strip, the quantities of papaverine HCl and of Viburnum required to produce in each instance a 50% decrease in amplitude of spontaneous contractions are estimated. On many tissues only a single dose of Viburnum produces quantitative responses, although some sedation is present after later doses. Papaverine sedation is usually reproducible before the administration of Viburnum; after Viburnum, papaverine tends to become more effective as a uterine sedative.

Using this method, we assayed a large number of commercial Viburnum prunifolium crude barks, also powdered extracts prepared from commercial barks under commercial conditions. Our studies on the barks indicated that a tentative standard might be established at a level of 500 γ of papaverine HCl corresponding to 1 Gm. of crude bark, when assays were made on an alcoholic extract prepared in accordance with a method in N. F. VII. Since the powdered extract represents four times the strength of the drug Viburnum in commercial practice, a tentative standard of 2000 γ of papaverine HCl/Gm. was established in this laboratory as a potency requirement for the powdered extract.

We have assayed a number of commercial Viburnum barks, many of which were identified pharmacognostically and found to be authentic by Dr. Heber W. Youngken. Many of these samples showed potencies of 500 γ/Gm. of bark. However, we have noted some samples showing much lower activities. As indicative of the current activity, the last seven samples tested have shown potencies, arranged in order of increasing strength, of 450, 500, 1035, 1125, 1565, 2335, and 2500 γ/Gm. of Viburnum bark. This is indicative of the trend of activities for Viburnum barks submitted for assay during 1947. Our laboratory test on samples submitted in 1944 and 1945 showed very few samples with a potency greater than 500 γ/Gm., and none over 1000 γ/Gm.

Similarly our early assays of the potency of the powdered extract of Viburnum indicated activities around 500 to 2000 γ/Gm. The improvement of potency of the powdered extracts has not been as noticeable as in the case of the crude drugs, submitted to us for bioassay. The last five samples of powdered extract submitted during 1947 for bioassay have shown values, arranged in order of progression, of 200, 1100, 2500, 2650, and 3000 γ/Gm. In some instances we had analyzed the crude Viburnum as well as the powdered extract prepared therefrom, and found that there was no consistent relationship in potency between the two. In fact some of the extracts were weaker than the crude drugs from which they had been prepared, when both samples were assayed on the same date. We are now starting detailed research in an attempt to learn the cause for such capricious results, and to determine the effect of various factors on the potency of powdered extracts prepared from the same crude drug on a laboratory and on a commercial scale.

CONCLUSIONS

1. The bioassay of Viburnum preparations against papaverine HCl for sedation of the isolated rat uterus has proved satisfactory.

2. As tentative standards 1 Gm. of authentic Viburnum prunifolium bark should correspond in sedative potency to 500 γ of papaverine HCl, and 1 Gm. powdered extract to 2000 γ.

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* Received Sept. 30, 1947 from the Munch Research Laboratories, Upper Darby, Pa.

Presented to the Scientific Section, A. PH. A., Milwaukee meeting, August, 1947.